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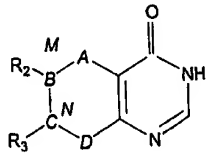
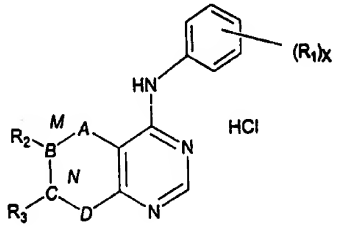
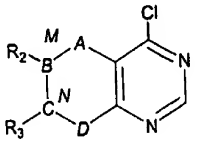
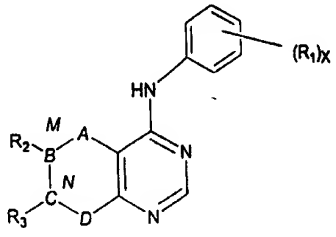
(54) Title: USE OF QUINAZOLINE DERIVATIVES FOR THE MANUFACTURE OF A MEDICAMENT IN THE TREATMENT OF HYPERPROLIFERATIVE SKIN DISORDERS









(57) Abstract

This invention is directed to methods and compositions for treating hyperproliferative skin disorders utilizing a quinazoline derivative as an active ingredient.

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DESCRIPTION

USE OF QUINAZOLINE DERIVATIVES FOR THE MANUFACTURE OF A MEDICAMENT IN THE TREATMENT OF HYPERPROLIFERATIVE SKIN DISORDERS

Field of the Invention

The present invention relates to methods, compositions and formulations for treating skin diseases and disorders characterized by keratinocyte hyperproliferation. In particular, the present invention relates to formulations of quinazoline compounds and processes for the manufacture of quinazoline derivatives.

Background of the Invention

Approximately four and one half million people in the U.S. are afflicted with psoriasis. Psoriasis is a skin disease often confined to localized areas of skin. It is typified by dry, scaly skin, abnormal thickening of epidermis, and rapid cell turnover in the skin. Psoriasis can be exacerbated by external factors including sun exposure, viral infections, and corticosteroid or beta-blocker use. Histologically, it is characterized by abnormalities including keratinocyte hyperplasia, abnormal differentiation sequence of keratinocytes in affected epidermis, and accumulation of leukocytes within the epidermis (Wright and Camplejohn, Eds., Psoriasis: Cell Proliferation, (Churchill Livingstone, Edinburgh, 1983), pp. 147-295; Weinstein, et al., J. Invest. Dermatol., 85:579, 1985).

Other skin disorders characterized by skin cell hyperproliferation include actinic keratoses, seborrheic keratoses and skin cancers such as basal cell carcinoma.

Infection with papilloma viruses also causes skin cell hyperproliferation (Zur Hausen, Int. Rev. Exp. Path., 25:307-326 (1983); Pfister, Rev. Phys. Biochem.

Pharmacol., 99:111-181 (1984). Infection of cervix by certain papilloma viruses has been strongly linked to a majority of cervical cancers, the second largest cause of cancer deaths in women worldwide (Parkin, *et al.*, Cancer, 41:184-187 (1988); Durst, *et al.*, Oncogene, 1:251-256 (1987); Broker and Botchan, Cancer Cells-DNA Tumor Viruses, New York: Cold Spring Harbor Laboratory, 1986).

Keratinocyte hyperplasia in psoriasis is linked to overproduction of cytokines such as TGFα and interleukin-6 (IL-6) and overexpression of epidermal growth factor receptor (EGF-R) in affected skin (Krueger, et al., J. Invest. Dermatol., 94:1355-1405, 1990). EGF-R is a 180-kD cell-surface receptor whose activity is regulated by both EGF and TGFα. In psoriasis vulgaris, EGF-R persists throughout the epidermis from the basal layers to the stratum corneum. Such persistent EGF-R has been shown to be biologically active *in vivo* in nude mice (Nanney, et al., J. Invest. Dermatol., 98:296-301, 1992).

Suggested treatment for psoriasis includes direct inhibition of keratinocyte growth and inhibition of activated lymphocyte proliferation (Dvir et al., J. Cell Biol. 113:857-865, 1991). Many topical products currently available are irritating, messy or simply ineffective. Topical steroids account for 90% of the psoriasis market in the United States and have many side effects including cutaneous atrophy, telangiectasia, formation of striae and tachyphylaxis.

Summary of the Invention

Epidermal hyperproliferation and angiogenesis are hallmarks of psoriasis. In the scope of the present invention it has been found that quinazoline derivatives such as 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline,

4-(3-Chlorophenylamino)-6-methylquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyanophenylamino)-6,7-dimethoxyquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6-methylquinazoline (identified as A1, A2, A3, A4, A5, and A6 in Table 1) have the properties of inhibiting or reducing the EGF-R tyrosine kinase activity and skin cell hyperproliferation. The administration of an effective amount of an above mentioned compound to a patient suffering from psoriasis or other hyperproliferative skin diseases will be able to inhibit the abnormal proliferation of skin cells, and decrease cornification, scaling or uneven thickness and other undesirable symptoms of psoriasis. The present invention is based on the aforementioned finding and is accordingly concerned with the novel use of the compounds in the treatment or prevention of skin diseases and disorders characterized by hyperproliferation.

Therefore, the present invention relates to methods and compositions for the treatment or prevention of hyperproliferative skin disorders, including, but not limited to, psoriasis (e.g. psoriasis vulgaris, psoriasis pustulosa, psoriasis erythrodermica, psoriasis arthropathica, parapsoriasis, palmoplantar pustulosis) and skin cancer. In preferred embodiments, a host (e.g. a mammal or human) is administered a composition containing a pharmaceutically effective amount of a quinazoline derivative of Formula V described in Figure 4e, such as A1, A2, A3, A4, A5, A6, or a pharmaceutically acceptable salt thereof. The compositions of this invention cure, reduce or prevent keratinocyte hyperproliferation or skin lesions in the host. A preferred drug is highly potent and selective with low toxicity.

In addition to psoriasis, other diseases or pathological conditions characterized by hyperproliferation and/or EGF-R overexpression and hyperactivity can be treated with the above-described compositions. These diseases and pathological conditions include, but are not limited to, keratinocyte proliferation and skin lesions caused or induced by Papilloma virus infection, seborrheic keratoses, acanthosis nigricans, ichthyosis (*e.g.* ichthyosis vulgaris and congenital ichthyoses), keratodermias, genodermatoses with pathological cornification disorders (*e.g.* Darier's disease), further lichen ruber planus, pityriasis rubra pilaris, and skin cancers such as basal cell carcinoma, squamous cell carcinoma and melanoma.

By "pharmaceutically effective" is meant the ability to cure, reduce or prevent one or more clinical symptoms of keratinocyte hyperproliferation, including, but not limited to, cornification, scaling, uneven thickness, inflammation, and rapid cell turnover in the skin.

The composition containing a pharmaceutically effective ingredient may be administered topically or systemically. In a preferred embodiment, it is administered topically to an affected skin area.

In a preferred embodiment, A1 or its pharmaceutically acceptable salts are used in the composition. Such a composition is especially suitable for topical treatment *vis-a-vis* systemic treatment of skin conditions because of the following properties of A1: (1) low toxicity, (2) short plasma half-life, and (3) relative high solubility in nonirritant solvents among anticancer compounds. Low toxicity is a desirable feature for a drug used to treat a non-life threatening disease. Short plasma half-life helps to keep the drug's therapeutic effects localized to where it is topically applied. Formulations with

nonirritant solvents alleviate the suffering of patients who have to use the drug repeatedly to receive the desired therapeutic effects.

In another aspect, this invention features a pharmaceutical composition for the treatment of a hyperproliferative skin disorder containing a pharmaceutically effective amount of a compound selected from the group consisting of A1, A2, A3, A4, A5, A6 and their pharmaceutically acceptable salts; and a pharmaceutically acceptable carrier. In a preferred embodiment, the pharmaceutical composition is for topical application to a host. In another preferred embodiment, the compound is selected from the group consisting of A1 and its pharmaceutically acceptable salts.

The composition may be in a unit dosage form or a multiple use dosage form. In a preferred embodiment, the composition is held within a container which includes a label stating to the effect that the composition is approved by the FDA in the United States (or an equivalent regulatory agency in a foreign country) for treating a hyperproliferative skin disorder such as psoriasis (*e.g.* psoriasis vulgaris, psoriasis pustulosa, psoriasis erythrodermica, psoriasis arthropathica, parapsoriasis, palmoplantar pustulosis), skin lesions caused by Papilloma virus infection, seborrheic keratoses, acanthosis nigricans, ichthyosis (*e.g.* ichthyosis vulgaris and congenital ichthyoses), keratodermias, genodermatoses with pathological cornification disorders (*e.g.* Darier's disease), further lichen ruber planus, pityriasis rubra pilaris, or a skin cancer such as basal cell carcinoma, squamous cell carcinoma or melanoma. Such a container provides a therapeutically effective amount of the active ingredient to be administered to a host.

In yet another aspect, this invention features a method of making a composition for the treatment of a hyperproliferative skin disorder by providing a pharmaceutically effective amount of a compound selected from the group consisting of A1, A2, A3, A4, A5, A6 and their pharmaceutically acceptable salts, and admixing the compound with a pharmaceutically acceptable carrier. Preferably, the composition is further packaged into a container in a unit dosage or a multiple use dosage.

This invention also features a method of preparing a compound for treating a hyperproliferative skin disorder by providing a plurality of compounds of Formula V, testing these compounds' ability to specifically inhibit EGF-R tyrosine kinase activity using the assays deseribed and disclosed in this application (such as those in Examples 1-8), and selecting those with activity in the range of A1, A2, A3, A4, A5, and A6. A pharmaceutically effective amount of such compounds is further packaged in a container with a pharmaceutically acceptable carrier.

Other features and advantages of the invention will be apparent from the following detailed description of the invention, and from the claims.

Brief Description of the Drawings

Figures 1(a), 1(b), 1(c), 1(d), 2(a), 2(b), 2(c) and 2(d) are general formulae of compounds that can be prepared with a process of this invention. Cl in Figures 1(b) and 2(b) may be replaced by another displaceable group which includes, but is not limited to, halogen, alkoxy, aryloxy and sulphonyloxy groups. Preferably, the displaceable group is chloro, bromo, methoxy, phenoxy, methanesulphonyloxy or toluene-*p*-sulphonyloxy.

Figures 3a, 3b and 3c are flow charts showing the general process for preparing compounds of the formulae in

Figures 1 and 2. Ethanol is used as solvent only as an example.

Figure 4a shows the structure of a compound of formula I.

Figure 4b shows the structure of a compound of formula II.

Figure 4c shows the structure of a compound of formula III.

Figure 4d shows the structure of a compound of formula IV.

Figure 4e shows the structure of a compound of formula V.

Detailed Description of the Preferred Embodiments

I. Quinazoline Derivatives of this Invention Target Egf-r for Therapeutic Intervention of Hyperproliferative Skin Diseases

This invention concerns treating or preventing psoriasis and other conditions characterized by keratinocyte hyperproliferation by inhibiting or reducing EGF-R activity with quinazoline derivatives.

Psoriatic epidermis is associated with altered protein tyrosine kinase, phospholipase C (PLC) and protein kinase C (PKC) activities, all of which are changed by chronic EGF-R activation. Growth factors, cytokines and their receptors are involved with the disease process of psoriasis (Krueger, et al., J. Invest. Dermatol., 94:1355-1405, 1990). Among them, EGF-R plays a crucial role in the hyperproliferation of keratinocytes in psoriatic lesions.

EGF-R is overexpressed in psoriatic keratinocytes (Nanney, et al., J. Invest. Dermatol., 86:260-265, 1986). The normal basilar distribution of EGF receptors in epidermal keratinocytes is markedly altered in *psoriasis*

vulgaris where they are also observed in the upper keratinocyte compartment (Nanney et al., J. Invest. Dermatol. 83:385-393, 1984; Green et al., J. Invest. Dermatol. 85:239-245, 1985). In a study on benign epidermal dermatoses, EGF receptor expression throughout the epidermis returned to a basal layer distribution when the lesion resolved (Ellis et al., N. Engl. J. Med. 317:1582-1587, 1987).

Psoriatic keratinocytes persistently secrete TGFα due to the overexpression of the TGFα gene (Gottlieb, et al., J. Exp. Med., 167:670-675, 1988; Elder, et al., Science, 243:811-814, 1989; Vassar, et al., Genes and Develop., 5:714-727, 1991). TGFα overexpression causes enhanced autocrine stimulation of the keratinocyte EGF-R (Grossman, et al., Proc. Natl Acad Sci USA, 86:6367-6371, 1989). IGF-1 receptor is also overexpressed in the psoriatic epidermis (Krane, et al., J. Invest. Dermatol., 96:419-424, 1991; Krane, et al., J. Exp. Med., 175:1081-1090, 1992). Activation by IGF-1 stimulates the synthesis of EGF-R and thus amplifies the effect of the EGF-R/TGFα autocrine loop. The finding that transgenic mice overexpressing TGFα in their skin develop lesions (Vassar, et al., Genes and Develop., 5:714-727, 1991) supports the crucial role of EGF-R in the pathogenesis of the disease.

An EGF-R has three functional domains: an extracellular ligand binding domain, a transmembrane domain and a cytoplasmic domain capable of phosphorylating tyrosine residues. Ligand binding to the extracellular ligand binding domain of membrane-bound EGF-R induces the formation of receptor dimers and allosteric changes that activate the intracellular kinase domain to result in the phosphorylation (autophosphorylation and/or transphosphorylation) of tyrosine residues. The phospho-tyrosine residues of the cytoplasmic domains of EGF-R then interact

with a host of cytoplasmic signaling molecules to activate signal transduction pathways (Ullrich and Schlessinger, Cell 61:203-212, 1990). During mitogenic activation of cells, the tyrosine kinase activity of EGF-R results in an increased activity of phospholipase C (PLC). The activation of EGF-R also results in the activation of protein kinase C (PKC), increased calcium uptake, and hydrolysis of membrane lipids to yield diacylglycerols (DAG) and inositol-triphosphate (IP3).

In psoriasis, hyperproliferation of keratinocytes is driven mainly by EGF-R and its ligands (Elder, et al., Science, 243:670-675, 1989). Some EGF-R blockers arrest the growth of psoriatic keratinocytes and are considered for clinical use (Ben-Bassat, et al., Exper. Dermatol., 4(2):82-84, 1995). Tyrosine kinase blockers of the tyrphostin family have been shown to block the proliferation of psoriatic keratinocytes grown in culture (Dvir, et al., J. Cell Biol., 113:857-865, 1991; Ben-Bassat, et al., Exp. Dermatol., 4:82-88, 1995). Tyrphostins inhibit EGF-R autophosphorylation and EGF-dependent tyrosine phosphorylation of intracellular target proteins in keratinocytes.

Skin cancers have also been associated with the expression of EGF-R ligands and anti-EGF-R antibodies have been shown to inhibit the growth of a skin cancer cell line expressing EGF-R. (See Hagedorn, M. and T. Baukrecht, Z. Hautkr 65(6):575-577, 1990; Kawamoto, T., *et al.* J. Biol. Chem. 259(12):7761-7766, 1984).

The present application shows that quinazoline derivatives of this invention, i.e., A1, A2, A3, A4, A5 and A6, are able to inhibit EGF-stimulated EGF-R phosphorylation. These compounds also are capable of inhibiting EGF-mediated skin cell growth *in vitro* and psoriatic skin cell proliferation *in vitro*. Specifically,

A1 potently inhibited ligand-induced autophosphorylation of the EGF receptor, and downstream signal transduction events, including DNA replication and cell cycle progression. The compound is specific for the EGF receptor, since it displayed little or no activity against unrelated receptor tyrosine kinases such as the receptors for platelet-derived growth factor or insulin-like growth factor-1. A1 was shown to block EGF receptor-dependent growth of tumor cells and fibroblasts engineered to overexpress EGF receptor. At micromolar concentrations, A1 was shown to inhibit the proliferation of keratinocytes isolated from psoriatic lesions. In skin penetration studies, radiolabelled compound penetrated human cadaver skin, reaching biologically effective concentrations in the epidermis within a 24-hour period.

II. Identification and Preparation of Compound

The chemical structures of A1, A2, A3, A4, A6 and A5 are shown below in Table 1. Methods for preparing these compounds can be found in U.S. Patent 5,457,105 issued October 10, 1995 and PCT publication WO 95/03283 published February 2, 1995, the totality of which is incorporated by reference herein.

In an example, A1 was prepared essentially as described in Barker, AJ. European Patent Application 0 566 226 A1, October 20, 1993; and Gazit et al., Bioorg. Med. Chem. 4:1203-1207, 1996. Briefly, methyl 2-amino-4,5-dimethoxybenzoate was treated with formamide at 180°C. The reaction was cooled and diluted with water. The precipitate was collected by filtration, washed with water and dried to give 6,7-dimethoxyquinazolone, which was treated with thionyl chloride and dimethylformamide at reflux, concentrated, and stirred with sodium bicarbonate solution. The resulting solid was collected and

crystallized from hexane to give 4-chloro-6,7-dimethoxyquinazoline, which was refluxed with 3-bromoaniline in ethanol, cooled, treated with 1 normal NaOH and the resulting solid collected by filtration to yield 4-(3-bromophenylamino)-6,7-dimethoxyquinazoline (A1).

II. Treatment Indications

The above identified compounds may be used to treat skin diseases or pathological conditions in mammals, especially in humans.

The hyperproliferative diseases which cause abnormal scaling and cornification of the skin include all forms of psoriasis, *e.g.* psoriasis vulgaris, psoriasis pustulosa, psoriasis erythrodermica, psoriasis arthropathica, parapsoriasis, palmoplantar pustulosis, all forms of ichthyoses, *e.g.* ichthyosis vulgaris and congenital ichthyoses, keratodermias of all types, *e.g.*, palmoplantar keratodermia, other genodermatoses with pathological cornification disorders, *e.g.* Darier's disease, further lichen ruber planus and pityriasis rubra pilaris.

In addition to psoriasis, other diseases or pathological conditions characterized by hyperproliferation and/or EGF-R overexpression and hyperactivity can be treated with the above-described compositions. These diseases and pathological conditions include, but are not limited to, keratinocyte proliferation and skin lesions caused or induced by Papilloma virus infection, seborrheic keratoses, acanthosis nigricans, ichthyosis (*e.g.* ichthyosis vulgaris and congenital ichthyoses), keratodermias, genodermatoses with pathological cornification disorders (*e.g.* Darier's disease), further lichen ruber planus, pityriasis rubra

pilaris, and skin cancers such as basal cell carcinoma, squamous cell carcinoma and melanoma.

V. Toxicity and Efficacy of Quinazoline Compounds

Toxicity and therapeutic efficacy of the above identified compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. A measure of the effectiveness and cell toxicity of a compound can be obtained by determining the therapeutic index: LD_{50}/IC_{50}. IC_{50}, the dose required to achieve 50% EGF-R tyrosine kinase activity inhibition, can be measured using standard techniques such as those described herein. LD_{50}, the dose lethal to 50% of the population, can be measured by standard techniques, such as using an MTT assay as described by Mossman, J. Immunol. Methods 65:55-63 (1983), by measuring the amount of LDH released as described by Korzeniewski and Callewaert, J. Immunol. Methods 64:313 (1983)and Decker and Lohmann-Matthes, J. Immunol. Methods 115:61 (1988), or by measuring the lethal dose in animal models. Another measure is the ratio LD_{50}/ED_{50}. ED_{50} is the dose therapeutically effective in 50% of the population. Compounds which exhibit large therapeutic indices are preferred. The therapeutic index should be greater than 2, preferably at least 10, more preferably at least 50.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC_{50} as determined in cell culture.

Plasma half-life and biodistribution of the drug and metabolites in plasma and major organs can be determined to facilitate the selection of drugs most appropriate for

the inhibition of a disorder. Such measurements can be carried out, for example, using HPLC analysis. Compounds that show potent inhibitory activity in the screening assays, but have poor pharmacokinetic characteristics, can be optimized by altering their chemical structure and retesting. In this regard, compounds displaying good pharmacokinetic characteristics can be used as models for the synthesis of other compounds.

Toxicity studies can also be carried out by measuring the blood cell composition. For example, toxicity studies can be carried out as follows: (1) the compound is administered to mice (an untreated control mouse should also be used); (2) blood samples are periodically obtained via the tail vein from one mouse in each treatment group; and 3) the samples are analyzed for red and white blood cells for indication of toxicity.

The data obtained from these cell culture assays and animal studies can be used in formulating a range of dosage for use in humans. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED_{50} with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

Materials and Methods

Cells and Cell Culture. All tumor cell lines were obtained from the American Type Culture Collection (Rockville, MD), unless otherwise specified. NIH3T3 mouse fibroblasts overexpressing the EGF receptor, IGF-1 receptor, insulin receptor, PDGF-b receptor, or a chimera of the EGF receptor extracellular domain fused to the Her-2 cytoplasmic domain were engineered using retroviral vectors. These cells will be referred to as 3T3-EGFR,

3T3-IGF1R, 3T3-IR, 3T3-PDGF-bR, and 3T3-EGFR/Her-2, respectively. All cell culture media and supplements were purchased from Gibco Life Technologies (Grand Island, NY) unless otherwise specified. All cells were grown in a humid atmosphere of 90-95% air and 5-10% CO_2 at 37°C. Cell lines were maintained under standard conditions in culture media recommended by the ATCC, unless otherwise specified. 3T3-EGFR, 3T3-PDGF-bR, and 3T3-EGFR/Her-2 cells were maintained in DMEM containing 10% calf serum (CS) and 2 mM GLN. 3T3-IGF1R and 3T3-IR cells were maintained in DMEM containing 10% FBS and 2 mM GLN. Keratinocytes were obtained from small biopsy specimens of split-thickness skin from patients with psoriasis and from healthy control donors. The biopsy specimens were treated to yield a population of cells enriched for keratinocytes, which were maintained in keratinocyte growth medium as described (Ben-Bassat et al., Exp. Dermatol. 4:82-88, 1995).

In vitro functional studies. The effect of A1 on receptor phosphorylation, DNA replication, cell cycle progression, and cell growth was studied. Cell lines examined in receptor phosphorylation ELISA were 3T3-EGFR, 3T3-EGFR/Her-2, 3T3-PDGF-bR, 3T3-IGF1R, and 3T3-IR. For analysis of DNA replication (measured as incorporation of BrdU) and cell cycle progression, 3T3-EGFR and 3T3-PDGF-bR cell lines were used. Cell growth studies were carried out on A431 (EGF-R+), BT474 (Her-2+), and C6 (PDGF-bR+) cell lines. The effect of A1 on the growth of human psoriatic keratinocytes was determined as previously described (Ben-Bassat et al., Exp. Dermatol. 4:82-88, 1995).

Skin Penetration. *In vitro* skin penetration studies were conducted using human cadaver skin. The vehicle formulation consisted of a petrolatum based topical

ointment containing 5.0% mineral oil, 3.0% glyceryl monostearate, 1.5% benzyl alcohol and 2.5% oleic acid. Six replicates of each A1 concentration (0.5%, 1.0%, 2.0%, and 4.0% drug) were evaluated during each study. The formulations were spiked with radiolabeled ^{14}C-A1 to obtain a specific radioactivity of 25 μCi/g. Radiolabeled ^{14}C-A1 was provided by SynPep Corp, Alameda, California (specific activity 5.89 mCi/mmol, lot #020196CL001). The formulation was applied (16.9 mg/cm^2) to human cadaver skin (1.77 cm^2 surface area and approximately 200 μm split thickness) mounted on a Franz static dffusion chamber. The chambers were filled with 4% BSA isotonic saline solution (6-10 mL reservoir volume) and equilibrated to a temperature of 37°C by a circulating pump. Percent penetration through the skin was determined by measuring cumulative radioactivity in the reservoir medium at the end of 24 hours or at regular intervals during the 24 hour period. Tissue distribution of the drug in the stratum corneum, epidermis and dermis was determined at the end of 24 hours. The stratum corneum was separated by tape stripping with cellophane tape until "glistening". The dermis and epidermis were separated by microwave technique as described (Kumar et al., Pharmaceutical Res. 6:740-741, 1989; Bronaugh, "Preparation of biological membranes", In: Methods for Skin Absorbtion, pp 61-66. Ed. Barbara Kempanien and William G. Reifenrath, CRC Press, 1990).

Example 1: Specific Inhibition of EGF-R Tyrosine Kinase Activity

EGF-R Whole Cell Kinase Assay

NIH3T3 clone C7 engineered to over-express human EGF-R and the human glioblastoma line U1242 that expresses PDGFR-beta were used for cellular kinase assays.

The activity of the above identified compounds in inhibiting EGF-stimulated EGF-R phosphorylation was measured in an ELISA assay.

EGF-R kinase activity (EGF-R-3T3 assay) in whole cells was measured as described below:

A. Pre-coat ELISA Plate

Coat ELISA plates (Corning, 96 well, Cat. #25805-96) with 05-101 antibody at 0.5 μg per well in PBS, 150 μl final volume/well, and store overnight at 4°C. Coated plates are good for up to 10 days when stored at 4°C.

On day of use, remove coating buffer and replace with blocking buffer (5% Carnation Instant NonFat Dry Milk in PBS). Incubate the plate, shaking, at room temperature (about 23°C to 25°C) for 30 minutes. Just prior to use, remove blocking buffer and wash plate 4 times with TBST buffer.

B. Seeding Cells

EGF-R/C7 cell line was used for this assay.

Choose dishes having 80-90% confluence for the experiment. Trypsinize cells and stop reaction by adding 10% CS DMEM medium. Suspend cells in DMEM medium (10% calf serum (CS) DMEM medium) and centrifuge once at 1000 rpm, and once at room temperature for 5 minutes.

Resuspend cells in seeding medium (DMEM, 0.5% bovine serum), and count the cells using trypan blue. Viability above 90% is acceptable. Seed cells in DMEM medium (0.5% bovine serum) at a density of 10,000 cells per well, 100 μl per well, in a 96 well microtiter plate. Incubate seeded cells in 5% CO_2 at 37°C for about 40 hours.

C. Assay Procedures

Check seeded cells for contamination using an inverted microscope. Dilute drug stock (10 mg/ml in DMSO) 1:10 in DMEM medium, then transfer 5 μl to a test well for a final drug dilution of 1:200 and a final DMSO concentration of 1%. Control wells receive DMSO alone. Incubate in 5% CO_2 at 37°C for one hour.

Prepare EGF ligand: dilute stock EGF in DMEM so that upon transfer of 10 μl dilute EGF (1:12 dilution), 25 nM final concentration is attained.

Prepare fresh HNTG* sufficient for 100 μl per well; and place on ice.

HNTG*:	10 ml
HNTG stock (5x)	2.0 ml
milli-Q H_2O	7.3 ml
EDTA, (100 mM, pH 7.0)	0.5 ml
Na_3VO_4, (0.5 M)	0.1 ml
Na_4PO_7, (0.2 M)	0.1 ml

After two hours of incubation with a drug, add prepared EGF ligand to cells, 10 μl per well, to yield a final concentration of 25 nM. Control wells receive DMEM alone. Incubate and shake at room temperature for 5 minutes.

Remove drug, EGF, and DMEM. Wash cells twice with PBS. Transfer HNTG* to cells, 100 μl per well. Place on ice for 5 minutes. Meanwhile, remove blocking buffer from other ELISA plate and wash with TBST as described above.

With a pipette tip securely fitted to a micropipettor, scrape cells from plate and homogenize cell material by repeatedly aspirating and dispensing the HNTG* lysis buffer. Transfer lysate to a coated, blocked, and washed ELISA plate. Incubate and shake at room temperature for one hour.

Remove lysate and wash 4 times with TBST. Transfer freshly diluted anti-phosphotyrosine (anti-Ptyr) antibody to ELISA plate at 100 μl per well. Incubate shaking at room temperature for 30 minutes in the presence of the anti-Ptyr antiserum (1:3000 dilution in TBST).

Remove the anti-Ptyr antibody and wash 4 times with TBST. Transfer the freshly diluted TAGO 30 anti-rabbit IgG antibody (anti-rabbit IgG antibody: 1:3000 dilution in TBST) to the ELISA plate at 100 μl per well. Incubate shaking at room temperature for 30 minutes.

Remove detection antibody and wash 4 times with TBST. Transfer freshly prepared ABTS/H_2O_2 solution to ELISA plate, 100 μl per well. Incubate at room temperature for 20 minutes. ABTS/H_2O_2 solution: 1.2 μl 30% H_2O_2 in 10 ml ABTS stock.

Stop reaction by adding 50 μl 5N H_2SO_4 (optional), and determine O.D. at 410 nm.

The maximal phosphotyrosine signal is determined by subtracting the value of the negative controls from the positive controls. The percent inhibition of phosphotyrosine content for extract-containing wells is then calculated after subtraction of the negative controls.

D. Materials & Reagents

1) EGF Ligand: stock concentration = 16.5 μM; EGF 201, TOYOBO, Co., Ltd. Japan.

2) 05-101 (UBI) (a monoclonal antibody recognizing an EGF-R extracellular domain).

3) Anti-Phosphotyrosine antibody (polyclonal) (made according to Fendley et al., Cancer Research 50:1550-1558, 1990).

4) TAGO antibody: Goat anti-rabbit lgG horse radish peroxidase conjugate, TAGO, Inc., Burlingame, CA.

5) TBST buffer:

Tris-HCl, pH 7.2,	50 nM
NaCl,	150 mM,
Triton X-100	0.1%

6) HNTG 5X stock:

HEPES	0.1 M
NaCl	0.75 M
Glycerol	50%
Triton X-100	1.0%

7) ABTS stock:

Citric Acid	100 rnM
Na_2HPO_4	250 mM
HCl, conc.	4.0 pH
ABTS*	0.5 mg/ml

*(2,2'-azinobis(3-ethylbenzthiazolinesulfonic acid).

Keep solution in dark at 4°C until use.

8) Stock reagents of:

EDTA	100 mM; pH 7.0
Na_3VO_4	0.5 M
Na_4PQ	0.2 M

Table 2 shows the activities of the quinazoline derivatives in the cellular EGF-R kinase assay and the ligand dependent cellular proliferation assay. Specifically, it shows the effectiveness of A1, A2, A3, A4, A6 and A5 in inhibiting EGF-R tyrosine kinase activity and curtailing EGF stimulated cell proliferation and the selectivity of the compounds. Table 2 shows IC_{50} (μM) of the above identified compounds. IC_{50} is the dose required to achieve 50% inhibition. In the order of decreasing activity, these compounds are A1, A4, A2, A3, A5 and A6.

To examine the effects of A1 on EGF-mediated receptor autophosphorylation, NIH 3T3 cells engineered to express EGF receptors (3T3-EGFR) were pretreated with titrated

doses of A1 prior to the addition of ligand. ELISA analysis showed that EGF-stimulated receptor phosphorylation was decreased in a dose-dependent manner. The average IC_{50} of six determinations was 13 nM.

To examine the effect of A1 on other receptor tyrosine kinases (RTK), NIH 3T3 cells engineered to overexpress various RTK were exposed to their corresponding ligands after pretreatment with A1, and the extent of ligand-stimulated receptor phosphorylation was determined by ELISA. As shown in Table 10, A1 inhibited receptor phosphorylation of the EGF receptor and a close relative, Her-2. However, other RTKs such as the platelet-derived growth factor receptor (PDGF-bR), the insulin-like growth factor receptor (IGF-1R) and the insulin receptor (IR) were not inhibited at concentrations up to 100 μM.

In Table 10, NIH 3T3 cells engineered to overexpress various RTKs were treated with corresponding ligand for 5 to 10 min following a 2 hr pretreatment with A1. Receptor phosphorylation was measured by ELISA. EGF-R/Her-2 denotes a chimeric receptor consisting of the extracellular (ligand-binding) domain of EGFR, and the transmembrane and cytoplasmic domains of Her-2.

Example 2: Inhibition of NIH 3T3 Cells Overexpressing EGF-R

Cellular Growth Assay

The efficacy of the above identified compounds in inhibiting or reducing EGF-R stimulated proliferation is measured by the 3T3 cell growth assay. The 3T3 growth assay was carried out as follows:

EGF-RC7 (NIH 3T3 C7 cells engineered to express EGF-R) and NIH 3T3C7 cells (as the control) were used for this

assay. NIH3T3C7 cells were seeded at 2500 cells/well, 10 μl/well in 10% CS + 2 mM Glutamine/DMEM, in a 96 well plate; EGF-RC7 cells were seeded at 6000 cells/well, 100 μl/well in 2% FBS + 2 mM Glutamine/DMEM, in a 96 well plate. Cells were incubated at 37°C, 5% CO_2 overnight to allow for cell attachment to the plate.

A quinazoline compound was added to the cells at day 2. The compound was prepared in the appropriate growth medium (10% CS + 2 mM glutamine in DMEM for NIH3T3C7 cells; 2% FBS+2 mM Glutamine in DMEM for EGF-RC7 cells) in a 96 well plate, and serially diluted. A total of 100 μl/well medium of the diluted compounds was added into the cells. The total volume of each well was 200 μl.

After the cells were treated with the compound for 4 days, the cells were washed with PBS and fixed with 200 μl/well ice-cold 10% TCA for one hour at 0-5°C.

Remove TCA and rinse wells 5 times with deionized water. Dry plates upside down with paper towels. Stain cells with 0.4% SRB at 100 μl/well for 10 minutes.

Pour off SRB and rinse plate 5 times with 1% acetic acid. Dry plate completely.

Solubilize the dye with 10 mM Tris-base at 100 μl/well for 10 minutes on a shaker.

Read the plate at dual wavelengths at 570 nm and 630 nm on Dynatech Elisa plate reader.

To determine whether the above identified compounds' inhibitory effect on EGF-R C7 cells is selective, the experiment described above was modified, replacing EGF-R C7 cells with human glioblastoma line U1242 that expresses PDGFR-beta.

The results of the assays described in Examples 1 and 2 are shown in Table 2. Example 1 demonstrates that the compounds of the invention are highly potent inhibitors of EGF-R enzymatic activity. Example 2 demonstrates that the

compounds of the invention are also highly potent inhibitors of EGF-R mediated cellular proliferation and that they are highly selective as well. The most potent and most selective compound is A1.

Materials and Reagents

(1) Dulbecco's Modified Eagle Medium (D-MEM), Gibco 511965-050;

(2) Calf serum, Gibco 16170-029;

(3) Trypsin-EDTA, Gibco 25200-056;

(4) Fetal Bovine Serum Certified, Gibco 16000-028;

(5) Dulbecco' 5 Phosphate-Buffered Saline (D-PBS), 10 Gibco 14190-029;

(6) Sulforhodamine B (SRB), Sigma 5-9012 0.4% SRB in 1% acetic acid;

(7) 10 mM Tris-base, Fisher BP152-5;

(8) 10% TCA, Trichroloacetic acid, Fisher A322-500;

(9) 96-well flat bottom plate (sterile), Corning 08-757-155;

(10) 100 ml reagent reservoir 9 (sterile), Matrix Technologies Corporation, 8086;

(11) Sterile pipet tips, Fisher 21-197-8E;

(12) 50 ml sterile TBST tubes, Fisher 05-539-6.

Example 3: Toxicity Studies

The toxicity of each of the compounds of the invention was evaluated by determining the LD_{10} and LD_{50} (the dose lethal to 10% or 50% of a population) in mice.

Briefly, female BALB/c mice (5 per group) were injected with a single dose of compound 1P in 50μl DMSO. Survival was measured at seven days. The results are presented in Table 3 below and demonstrated that the compounds of the invention are relatively non-toxic.

Multiple dose toxicity studies were also conducted. A1 in DMSO was administered IP to Balb/c nude mice at doses ranging from 5 to 30 mg/kg/day for 17, 20 or 30 days. The results are shown below:

Exp	Dose	Duration	% Mortality
1	30	17	0
2	5	20	0
3	10	20	0
4	30	20	0
3	30	33	25

Up to 12.5% mortality can periodically be observed with prolonged treatment (33 days) with vehicle control alone.

Example 4: Skin Penetration and Absorption Studies

Three formulation categories (nine formulations total) consisting of Petrolatum Ointments, Emollient Creams and Polyethylene Glycol Ointments were developed for the skin penetration studies (Tables 6 and 7). The drug concentration was 2.0% for all formulations and time points were drawn at 0, 1 and 24 hours. There was an immediate turnaround of these results which indicated that the petrolatum ointments and the emollient creams showed approximately ten fold increase in penetration as compared to the PEG ointment with the percents listed in Tables 7 and 8.

Plasma levels of A1 were measured in nu/nu mice. Approximately 60 mg of a 2% (w/w/ointment was applied to the backs of four female mice using a stainless steel spatula. The dose was spread evenly over the back surface of each mouse.

Plasma was prepared from blood samples taken 30 or 60 min. following application (2 mice per time point) and examined by HPLC. In a second experiment, approximated .50 mg of the 2% (w/v) ointment was applied in the same manner. Blood samples were taken at 15 and 30 min and at 1, 2.25 and 30 hours. The mice exhibited no discomfort or increased grooming behavior for the duration of the experiments. Only trace (<2μg/ml) amounts of A1 were detected at 30 min. or later time points. The lower limit of quantitation for the HPLC assay is 1.0μg/ml.

Based on the above described studies, a topical formulation of A1 is prepared (see Table 9).

In another example, skin penetration studies were carried out using a petrolatum-based formulation containing 0.5, 1.0, 2.0 and 4.0% of A1. The results are summarized in Table 12. These data show that the amount of A1 recovered in the epidermis increased with the concentration applied to the skin and did not reach saturation at 4.0% A1. Furthermore, 0.5% A1 applied topically to human cadaver skin resulted in a concentration in the epidermis after 24 hours ranging from 141-355 μM. This is 10 to 25 fold more than the IC_{99} for inhibition of psoriatic keratinocyte proliferation (by extrapolation of the growth inhibition curve, the IC_{99} was estimated to be 14 μM on day 5). Thus, biologically relevant concentrations of the drug penetrated the skin and reached the target tissue (epidermis) within 24 hours of application.

Calculations to determine micromolar were made assuming that 100 microns of the skin section (200 microns total) represented epidermis. The area of the cadaver skin used was 1.77 cm^2. The calculated volume using this assumption is 17.7 μL. Molar concentration was calculated from the microgram recovery in the epidermis.

Example 5: Inhibition of EGF-driven DNA replication

Autophosphorylation of receptor tyrosine kinases such as the EGF-R initiates a signaling cascade that results in nuclear changes in the cell, including DNA replication and entry into the S-phase of the cell cycle. Therefore, the effect of A1 on ligand-induced DNA replication was studied. 3T3-EGFR cells and 3T3-PDGF-bR cells were stimulated with the corresponding ligand in the presence of titrated doses of A1. After 20 hours, DNA replication was determined by measuring the incorporation of bromodeoxyuridine (BrdU). EGF-driven DNA replication in 3T3-EGFR cells was significantly inhibited by A1 in a dose-dependent manner, with an IC_{50} of 30 nM. In contrast, PDGF-driven DNA replication in 3T3-PDGF-bR cells was inhibited only by much higher concentrations of A1 (IC_{50} = 10 μM).

Example 6: A1 inhibits EGF-driven cell cycle progression

A1 was examined for its ability to inhibit receptor-stimulated cell cycle progression. 3T3-EGFR and 3T3-PDGF-bR cells were incubated with drug and ligand (EGF and PDGF, respectively) for 20 hours. The percentage of cells in various phases of the cell cycle was determined by propidium iodide staining and fluorescence activated cell sorting (FACS) analysis. Ligand stimulation of each cell line resulted in an increase in the percentage of cells in the S-phase (4% in resting cells, 52% in PDGF-stimulated, 72% in EGF-stimulated). Similar to the results seen in receptor phosphorylation and DNA synthesis assays, the addition of A1 inhibited cell cycle progression much more potently in the EGF-driven cells than in the PDGF-driven cells (IC_{50} = 34 nM [EGF], 4.1 μM [PDGF]). These data demonstrate that the inhibition of

EGF-R autophosphorylation by A1 results in inhibition of downstream effects, suggesting that A1 might specifically inhibit EGF-driven cellular proliferation.

Example 7: A1 inhibits cellular proliferation driven by the EGFR/Her family of RTKs

Since EGF receptor signaling and cell cycle progression were inhibited by A1, the effect of the compound on cellular proliferation was also examined. Cells expressing various receptor tyrosine kinases were seeded overnight in 10% FBS and A1 was added in titrated doses the next day. After 4 days, cell density was measured by SRB staining. IC_{50} values were determined by regression analysis. For these experiments, cell lines whose growth is dependent on naturally-expressed RTK were examined. Cellular proliferation was measured by sulforhodamine B (SRB) staining after a 4 day incubation in the presence of titrated doses of A1, and IC_{50} values were calculated. The data in Table 11 clearly show that A1 preferentially inhibited the growth of cell lines driven by the EGF-R/Her family of RTK, but did not inhibit PDGF-bR-dependent cell growth.

Example 8: A1 inhibits growth of psoriatic human keratinocytes

Keratinocytes were obtained from psoriasis patients after informed consent, and the effect of A1 on the growth of the keratinocytes was examined as described by Ben-Bassat et al., Exp. Dermatol. 4:82-88, 1995. A1 inhibited the growth of psoriatic keratinocytes, with an IC_{50} of < 1.0 μM on days 5 through 12 of culture. These results indicate that blocking EGF-R signaling is sufficient to inhibit the proliferation of psoriatic keratinocytes.

V. Formulations and Administration

The compounds of the present invention can be administered to a host alone, or in a pharmaceutical composition comprising the active compound and a carrier or excipient. The compounds also can be prepared as pharmaceutically acceptable salts. Examples of pharmaceutically acceptable salts include acid addition salts such as those containing hydrochloride, sulfate, phosphate, sulfamate, acetate, citrate, lactate, tartrate, methane sulfonate, ethane sulfonate, benzene sulfonate, *p*-toluene sulfonate, cyclohexylsulfamate and quinate (e.g. those disclosed in PCT/US92/03736 and PCT/GB94/01544, incorporated by reference herein). Such salts can be derived using acids such as hydrochloric acid, sulfuric acid, phosphoric acid, sulfamic acid, acetic acid, citric acid, lactic acid, tartaric acid, malonic acid, methanesulfonic acid, ethanesulfonic acid, benzenesulfonic acid, *p*-toluenesulfonic acid, cyclohexylsulfamic acid, and quinic acid. Pharmaceutically acceptable salts also include alkali metal salts such as sodium or potassium salts, alkaline earth metal salts such as calcium or magnesium salts, ammonium salts or salts with an organic base which afford a physiologically-acceptable cation such as salts with methylamine, dimethylamine, trimethylamine, piperidine, morpholine or tris-(2-hydroxyethyl)amine.

Pharmaceutically acceptable salts can be prepared by standard techniques. For example, the free base form of the compound is first dissolved in a suitable solvent such as an aqueous or aqueous-alcohol solution containing the appropriate acid. The salt is then isolated by evaporating the solution. In another example, the salt is prepared by reacting the free base and acid in an organic solvent.

In accordance with this invention the aforementioned quinazoline derivatives can be administered to a subject for reducing or inhibiting keratinocyte proliferation. The compounds are useful as a prophylaxis or means for treating disorders such as psoriasis. The pharmaceutical compositions of the invention contain the compounds in association with a compatible pharmaceutically acceptable carrier material.

Techniques for formulation and administration may be found in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Co., Easton, PA (1990). Any conventional carrier material can be utilized. The carrier material can be an organic or inorganic carrier material suitable for topical, enteral, percutaneous or parenteral administration. Carriers or excipients can be used to facilitate administration of the compound, for example, to increase the solubility of the compound. Suitable carriers and excipients include, but are not limited to, water, ethanol, polysorbate-80, triacetin, gelatin, gum arabic, lactose, starch, magnesium stearate, talc, vegetable oils, polyalkyleneglycols, petroleum jelly, benzyl alcohol, polyethylene glycols (e.g. PEG-300 and PEG-400), propylene carbonate, propylene glycol, Transcutol, Petrolatum, vegetable oils, mineral oil, stearyl alcohol, Laureth-4, calcium carbonate, calcium phosphate, various sugars or types of starch, cellulose derivatives and mixtures thereof. Furthermore, the pharmaceutical preparations may contain other pharmaceutically active agents. Additional additives such as flavoring agents, preservatives, stabilizers, emulsifying agents, buffers and the like may be added in accordance with accepted practices of pharmaceutical compounding.

Table 4 lists the excipients used for solubility testing of A1. The amount of compound for each test was between 1 to 15 mg/mL unless otherwise indicated. Visual solution clarity was recorded immediately and 24 Hrs. after rocking at room temperature in a type I glass container. Table 5 shows the solubility of A1 in more excipients.

The pharmaceutical preparations can be made up in any conventional form including: (a) a solid form for oral administration such as tablets, capsules, pills, powders, granules, and the like; (b) a liquid form for oral administration such as solutions, syrups, suspensions, elixirs and the like; (c) preparations for parenteral administration such as sterile solutions, suspensions or emulsions; and (d) preparations for topical administrations such as solutions, suspensions, ointments, creams, gels, micronized powders, aerosols, aqueous gels, Petrolatum ointments, PEG ointments and the like. The pharmaceutical preparations may be sterilized and/or may contain adjuvants such as preservatives, stabilizers, wetting agents, emulsifiers, salts for varying the osmotic pressure and/or buffers.

For topical administration to the skin the aforementioned compounds are preferably prepared as ointments, tinctures, creams, gels, solutions, lotions, sprays, suspensions, shampoos, hair soaps, perfumes and the like. In fact, any conventional composition utilized for application to the scalp or skin can be utilized in accordance with this invention. Preferred formulations include gels, lotions and creams. The pharmaceutical preparation for topical administration to the skin can be prepared by mixing the aforementioned active ingredient (i.e., a pharmaceutically effective amount of a compound)

with non-toxic, therapeutically inert, solid or liquid carriers customarily used in such preparations.

In preferred embodiments, these preparations contain at least about 0.0005 percent by weight, of the active ingredient based upon the total weight of the composition. The active ingredient, the compound, may be used in topical compositions in amounts significantly exceeding 10 percent. It is preferred that these preparations contain about 0.01 to 10 percent by weight of the active ingredient based upon the total weight of the composition.

It is also preferred that these preparations are applied once or twice daily to the skin. These preparations can be applied according to the need of the patient. In carrying out this invention, the active ingredient can be applied in an aqueous solution or an alcohol solution such as ethyl alcohol.

In preparing the topical preparations described above additives such as preservatives, thickeners, perfumes and the like conventional in the art of pharmaceutical compounding can be used. In addition, conventional antioxidants or mixtures of conventional antioxidants can be incorporated into the topical preparations containing the aforementioned active agent. Among the conventional antioxidants which can be utilized in these preparations are included N-methyl-α-tocopherolamine, tocopherols, butylated hydroxyanisole, butylatedhydroxytoluene, ethoxyquin and the like. Cream-base pharmaceutical formulations containing the active agent, used in accordance with this invention, are composed of aqueous emulsions containing a fatty acid alcohol, semi-solid petroleum hydrocarbon, 1,2-ethyleneglycol and an emulsifying agent.

Ointment formulations containing the active agent in accordance with this invention comprise admixtures of a

semi-solid petroleum hydrocarbon with a solvent dispersion of the active material. Cream compositions containing the active ingredient for use in this invention preferably comprise emulsions formed from a water phase of a humectant, a viscosity stabilizer and water, an oil phase of fatty acid alcohol, a semisolid petroleum hydrocarbon and an emulsifying agent and a phase containing the active agent dispersed in an aqueous stabilizer-buffer solution. Stabilizers may be added to the topical preparation. Any conventional stabilizer can be utilized in accordance with this invention. In the oil phase, fatty acid alcohol components function as a stabilizer. These fatty acid alcohol components are derived from the reduction of a long-chain saturated fatty acid at least about 14 carbon atoms. Also, conventional perfumes and lotions generally utilized in topical preparation for the hair can be utilized in accordance with this invention. Furthermore, if desired, conventional emulsifying agents can be utilized in the topical preparations of this invention.

Parenteral dosage forms can be infusions or injectable solutions. Such dosage forms can be injected, e.g., intravenously, subcutaneously or intramuscularly. These preparations can also contain other medicinally active substances. In preferred embodiments, a daily dosage of from about 0.01 mg to about 2 mg per Kg of body weight is utilized in parenteral formulations. In further preferred embodiments, a daily dosage of from about 0.025 mg to about 0.5 mg per kg of body weight of the patient is utilized. Additional additives such as flavoring agents, preservatives, stabilizers, emulsifying agents, buffers and the like may be added in accordance with accepted practices of pharmaceutical compounding.

A preferred oral dosage form comprises capsules of hard or soft gelatin methylcellulose or of another

suitable material easily dissolved in the digestive tract. The enteral dosages contemplated in accordance with the present invention will vary in accordance with the needs of the individual patient as determined by the prescribing physician. In preferred embodiments a daily dosage of from about 0.01 mg. to about 2 mg per Kg of body weight is utilized. In further preferred embodiments a daily dosage of from about 0.025 mg to about 0.5 mg per kg of body weight of the patient is utilized. This dosage may be administered according to any dosage schedule determined by the physician in accordance with the requirements of the patient.

It is likewise within the purview of the present invention to incorporate the therapeutically active substances enumerated herein in any desired amount for enteral administration within the oral unit dosage form. It is preferred, however, to formulate preparations containing the active substance of the present invention in such a manner that each dose contains from about 0.05 mg to about 100 mg, particularly from about 0.1 mg to about 10 mg of the active substance with suitable therapeutically inert fillers and diluents. It is especially preferred to incorporate such a dosage into soft gelatin capsules and tablets.

A. Topical Formulations

The present invention is concerned with novel formulations of quinazoline, quinazoline derivatives, and pharmaceutically acceptable salts thereof which are suitable for topical application for treatment of psoriasis and other skin diseases.

Topical formulations include ointments, tinctures, creams, gels, solutions, lotions, sprays, suspensions, shampoos, hair soaps, perfumes and so on. It is an object

of the present invention to provide a vehicle for quinazoline, quinazoline derivatives, and pharmaceutically acceptable salts thereof. Such vehicle possesses one or more of the following characteristics:

1. Non-irritating or low-irritating, and non-allergenic or low-allergenic to skin.
2. High capacity in storing pharmaceutically effective amount of quinazoline, quinazoline derivatives, and pharmaceutically acceptable salts thereof.
3. Physically and chemically stable at ambient temperature.
4. Efficient drug release and delivery, good skin penetration.

In that regard, this invention generally features a topical formulation containing a pharmaceutically effective amount of quinazoline, a quinazoline derivative, or a pharmaceutically acceptable salt thereof dispersed in a nonpolar hydrocarbon mixture composed of compounds made of carbon and hydrogen, and an excipient which in combination with the nonpolar hydrocarbon mixture enhances the penetration of quinazoline or its derivative through the skin. Preferably, the nonpolar hydrocarbon mixture is a petrolatum ointment, including, but not limited to, white petrolatum USP ointment. Other nonpolar hydrocarbon mixtures include, but are not limited to, mineral oil USP, light mineral oil NF, paraffin NF, synthetic paraffin NF, squalane NF, microcrystalline was NF, hexane, isohexane, heptane, decane, decene, decyne, octadecane, benzene, toluene, naphthalene, polyethylene, polypropylene, and polystyrene.

The concentration of quinazoline or its derivative in the topical formulation is preferably from about 0.01% to about 10.0% w/w, more preferably from about 0.1% to about

4.0% w/w, and even more preferably from about 0.25% to about 1.0% w/w.

Quinazoline or its derivative in the topical formulation may be micronized to facilitate suspension in the ointment, dispersion and/or skin absorption. Various known methods of particle size reduction may be utilized to reduce the size of the quinazoline particles. Preferably, the majority of the particles have a maximum dimension (i.e., diameter) of no more than about 50 microns, and more preferably 90% or more of the particles have a maximum dimension of no more than about 20 microns.

An excipient which increases the percentage of quinazoline or its derivative that reaches dermis and epidermis (as measured by % penetrated to reservoir, dermis, epidermis and stratum corneum (R + D + E + SC)) is selected for the topical formulation of this invention. A method for selecting excipients is described in Example 2 in the Detailed Description of the Preferred Embodiments. Preferably, the excipient increases % penetrattion (R + D + E + SC) by more than fifty percent. Examples of skin penetration enhancer include, but are not limited to, low molecular weight alcohol such as benzyl alcohol, NF, and unsaturated fatty acid such as oleic acid, NF, or unsaturated fatty alcohol. The unsaturated fatty acid or unsaturated fatty alcohol preferably has at least one double bond and no fewer than twelve carbons. Other excipients disclosed or described in Percutaneous Penetration Enhancers, CRC Press (E. W. Smith and H. I. Maibach ed. 1995, ISBN 0-8493-2605-2) as enhancing skin penetration of drugs can be used in the formulations of this invention as well, especially those described in Chapter 9.1 -- "Fatty Acids as Skin Permeation Enhancers". The entire content of the book is incorporated by

reference herein. Excipients may be used alone or in combination with each other in the topical formulation.

Benzyl alcohol may be used in a concentration from about 0.050% to about 5.0% w/w, preferably no more than about 3.0% w/w, and more preferably no more than about 1.0% w/w.

Oleic acid may be used in a concentration from about 0.050% to about 10.0% w/w, preferably no more than about 5.0% w/w, and more preferably no more than about 2.5% w/w.

An antioxidant may be used in combination with oleic acid to reduce oxidation damage. Candidate antioxidants include, but are not limited to, butylated hydroxytoluene (BHT), NF, butylated hydroxyanisole (BHA), ascorbic acid (Vitamin C), hydroquinone, ascorbyl palmitate, acetyl cysteine, N-methyl-α-tocopherolamine,, ethoxyquin, nordihydroguaiaretic acid (NDGA), sodium bisulfite, propyl gallate and α-tocopherol (Vitamin E). They may be used in a concentration from about 0.001% to about 1.0% w/w. In the case of BHT, preferably it is used in a concentration from about 0.0005% to about 0.1% w/w, and more preferably no more than 0.01% w/w.

Inert ingredients which stabilize the petrolatum suspension may be added, including, but not limited to, an emollient stabilizer (e.g. mineral oil) and an nonionic emulsifier (e.g. glyceryl monostearate).

Optionally, minor amounts of dyes, perfumes, sunscreens, or other agents which are commonly used in topical pharmaceutical compositions may be added. Furthermore, such topically active medicaments as the anti-inflammatory corticosteroids and antimicrobials may also be incorporated.

Preferably, A1 or a pharmaceutically acceptable thereof is used in the composition. Such a composition is especially suitable for topical treatment *vis-a-vis*

systemic treatment of skin conditions because of Al's low toxicity and short plasma half-life. Low toxicity is a desirable feature for a drug used to treat a non-life threatening disease. Short plasma half-life helps to keep the drug's therapeutic effects localized to where it is topically applied.

The formulation may be in a unit dosage form or a multiple use dosage form. In a preferred embodiment, a formulation of this invention is held within a container which includes a label stating to the effect that the composition is approved by the FDA in the United States (or an equivalent regulatory agency in a foreign country) for treating a hyperproliferative skin disorder such as psoriasis (*e.g.* psoriasis vulgaris, psoriasis pustulosa, psoriasis erythrodermica, psoriasis arthropathica, parapsoriasis, palmoplantar pustulosis), skin lesions caused by Papilloma virus infection, seborrheic keratoses, acanthosis nigricans, ichthyosis (*e.g.* ichthyosis vulgaris and congenital ichthyoses), keratodermias, genodermatoses with pathological cornification disorders (*e.g.* Darier's disease), further lichen ruber planus, pityriasis rubra pilaris, or a skin cancer such as basal cell carcinoma, squamous cell carcinoma or melanoma. Such a container provides a therapeutically effective amount of the active ingredient to be administered to a host.

In another aspect, this invention features a method of administering quinazoline, a quinazoline derivative, or a pharmaceutically acceptable salt thereof topically by applying a topical formulation described above. Generally, disease-inflicted skin is applied with a topical formulation containing a pharmaceutically effective amount of quinazoline, a quinazoline derivative, or a pharmaceutically acceptable salt thereof dispersed in a nonpolar hydrocarbon mixture, and an excipient which in

combination with the nonpolar hydrocarbon mixture enhances the penetration of quinazoline or its salt through skin. Preferably, the nonpolar hydrocarbon mixture is a petrolatum ointment or mineral oil.

Formulation Series I

Ointment formulations containing the active agent comprise admixtures of a semi-solid petroleum hydrocarbon with a dispersion of the active material in an inert liquid ingredient.

Cream-base pharmaceutical formulations are composed of oil-in-water emulsions containing a wide variety of excipients, including, but not limited to, fatty alcohol, semi-solid petroleum hydrocarbon, propylene glycol or 1,2-propanediol. Cream compositions containing the active ingredient preferably comprise emulsions formed from a "water" phase containing one or more humectants, a viscosity stabilizer, a preservative and water, an "oil" phase containing one or more fatty alcohols, a semisolid petroleum hydrocarbon, and one or more emulsifying agents. Stabilizers may be added to the topical preparation.

The petrolatum formulations initially developed are shown in Table 13. These formulations were put on a three month stability screening protocol as described in Example 9. The petrolatum formulations were also sent for *in vitro* skin penetration studies with human cadaver skin; six replicates were run for each formulation. A summary of the test protocol is given in Example 10 and a summary of the skin penetration results is given in Table 13.

The drug concentration was equal in all the formulations tested. Petrolatum ointments and emollient creams containing dispersed drug substance show substantially better drug delivery in terms of micrograms delivered and percent of dose delivered than PEG ointments

containing dissolved drug substance (Creams and PEG ointments are not shown in Table 13). In comparing the two petrolatum ointments and the three creams, they are all equal considering test error. The petrolatum ointments containing suspended drug showed total skin penetration (R+D+E+SC) of about 4% and 6%. Formulation F1 delivered 47 micrograms to R+D+E+SC, at an efficiency of 7.9%.

Formulation F2 also gave good penetration at 34 micrograms (5.6%) delivered to (R+D+E+SC). The lack of a penetration enhancer in this formula probably accounts for its lower delivery compared to F1.

Formulation Series II

A second series of five petrolatum formulations for skin penetration evaluation are shown in Table 14. This group consists of the formulation from Series I showing the highest penetration (F1) and four new petrolatum ointments which were variations of F1 designed for improved penetration. Four new cream formulations were also prepared which were variations of the best cream from Series I designed for improved penetration (not shown in Table 14).

The mineral oil used in the petrolatum ointment formulations was changed from NF grade (Light Mineral Oil, NF) to USP grade (Mineral Oil, USP). The USP grade has a higher viscosity. The USP grade was used in all subsequent work.

Because oleic acid is susceptible to oxidation due to its C=C bond, 0.2% alpha tocopherol (Vitamin E, USP) was added to several formulations (see Table 14). However, it was found that this high level of tocopherol caused a darkening of the color of the formulated product after two weeks at 40°C. In the alternative, a lower level of

tocopherol or approximately 10 ppm (0.001%) BHT is added to the petrolatum ointment formulation to help prevent possible oleic acid oxidation.

The penetration results of the petrolatum formulations are shown in Table 14. The petrolatum ointments (except F6) gave substantially better penetration than the creams in this series. Due to the large errors present in this type of test, a general rule is that a significant difference exists between two formulations if there is at least a 2-fold difference between results. In this case, the average penetration difference between the four best ointments and the four creams is a factor of about 3-fold.

Among petrolatum formulations, formulation F1 again gave the best penetration results of all the formulations tested, delivering 44 micrograms to (R+D+E+SC), at an efficiency of 7.4%.

Formulation F5, containing 2.5% oleic acid and 1.5% benzyl alcohol, also gave very good penetration at 36 micrograms (6.1%) delivered to (R+D+E+SC). This result can be considered equal to F1. Because oleic acid is a potential irritant, this formulation is not as irritant as F1.

Formulations F3 and F4 were modifications of F1 containing additional excipients (benzyl alcohol and laureth 4) which have relatively high drug solubility. These formulations did not perform as well as F1; the reason may be that addition of a good solvent to a suspended drug formulation favors the drug remaining in the formulation rather than moving toward and through the skin.

Formulation F6, containing 5% propylene glycol (PG, a penetration enhancer), gave poor penetration results. This may be because PG is not miscible with petrolatum and

forms a two-phase system. In this case, the PG, which shows very low drug solubility, may have interfered with the penetration enhancing properties of the oleic acid.

Overall, the creams did not perform as well in this study (maximum 2% penetration) as they did in Series I, while the petroleum ointments continued to show excellent penetration (up to 6%). Therefore, petrolatum was chosen as the preferred vehicle, and another penetration experiment was performed to optimize the formulations.

Formulation Series III

The third series of eight petrolatum formulations for skin penetration evaluation is shown in Table 15. This series was designed to study the effects of varying the drug concentration, and also for optimization of the concentrations of the excipients benzyl alcohol and oleic acid. The penetration results are shown in Table 15.

One cream formulation was prepared as well, which contains (%):

A1	2.00
WHITE PETROLATUM, USP	5.00
BENZYL ALCOHOL, NF	2.00
STEARYL ALCOHOL, NF	10.00
OCTYLDODECANOL, NF	5.00
BRIJ721	2.00
BRIJ72	2.40
PROPYLENE GLYCOL, USP	5.00
METHYLPARABEN, NF	0.20
HYDROXYETHYL CELLULOSE, NF	0.30
PURIFIED WATER, USP	66.10

The penetration result of the cream formulation is 3.6% (R+D+E+SC).

Overall, the cream did not perform as well in this study as it did in Series I, while the petrolatum ointment continued to show good drug penetration into the skin layers. All the petrolatum ointments at 2% drug gave substantially better penetration than the cream at 2% drug, a conclusion drawn also from Series II and confirmed in Series III.

The petrolatum ointment control sample in this test, F10, was identical to F5 in Series II except that the Series II formula also contained 0.2% tocopherol, which should not have substantially affected penetration. The results for F10 in this test are very close to those of F5: only a 2 microgram difference in total reservoir + dermis + epidermis + stratum corneum (R+D+E+SC) penetration.

Among petrolatum ointments, the drug concentration dose study gave essentially a linear response over the range of 0.5 to 4.0% A1 (rounding off the R+D+E+SC penetration to the nearest 5 micrograms gives an exactly linear result of 80, 40, 20 and 10 micrograms). The formula at 4% drug therefore gave about double the microgram penetration of the best of the 2% drug formulas. This would allow drug concentration to be varied to meet a given target delivery, with good predictability.

Comparing F10, F11, and F12 gives the effect of varying the oleic acid concentration from 2.5 to 1.25 to 0% in the same base formula. Looking at the R+D+E+SC penetration implies that 2.5% is equal to 1.25%, and these are probably better than 0%. The 0% formula still gave fair penetration probably because the benzyl alcohol was maintained at 1.5%.

Comparing F10, F13, and F14 gives the effect of varying the benzyl alcohol concentration from 1.5 to 0.75 to 0% in the same base formula. The results of this

comparison show that 0.75% and 0% are equal, while 1.50% may be slightly worse. In any case, the differences are relatively small, similar to the oleic acid dose study. This may imply a synergistic effect between the oleic acid and the benzyl alcohol. Since F12 gave the lowest penetration results of the 2% ointments, oleic acid is probably more important to penetration than benzyl alcohol.

Overall, most of the 2% ointment results are relatively close. F11 and F13 gave the highest R+D+E+SC penetration. The physical stability results showed that the latter might possess slightly better stability.

Because the drug substance is present as a solid suspension in the formulated ointment, it was micronized for the formulations to be used in toxicology studies and clinical studies. Micronization produces smaller particles and increases the total drug substance surface area and dispersion of the suspension in the ointment; these characteristics may result in more efficient skin penetration of the drug substance. Drug micronization contributes to better characterization and definition of drug substance particle size for future bioequivalence needs. The drug micronizing process also improves the cosmetic elegance of the formulation as an emollient by eliminating palpable detection of solids in the ointment.

The micronized drug substance was tested by HPLC which indicated that the micronization did not reduce the drug's purity.

F1, F11 and F13 were screened for stability with HPLC. F1 and F13 are stable at ambient temperature for at least three months. F11 is stable at ambient temperature for at least two months.

An additional exemplary petrolatum formulation is described in Table 16.

All the excipients in the selected formulation are compendial. A manufacturing process is described in Example 11.

Example 9: Physical Stability Screening

Method:

A 5 to 15 gram sample is placed in a 20 ml glass scintillation vial and capped with a urea screw cap having a PE cone liner.

Storage Conditions:

50°C (45 to 55°C)
40°C (37 to 42°C)
30°C (27 to 32°C)
Ambient Room Temperature (15 to 30°C)
Refrigerator (2 to 8°C)
Freezer (-10 to -30°)
Freeze-thaw cycling (1 week at freezer followed by 1 week at ambient per cycle)

Evaluation Times:

1 week (optional), 2 weeks (optional), 1 month, 2 months & 3 months.
The 50°C sample is typically terminated at 1 month. Samples at any test station may be terminated based on gross instability or other pertinent reason.

Evaluation:

Evaluation is by physical examination for general appearance, color, odor, consistency/viscosity, separation/syneresis, etc.

Example 10: In-vitro Skin Penetration Study Protocol

Set-up: Franz static diffusion chambers are filled with a 4% BSA isotonic buffered saline solution (6-10 mL reservoir volume) and equilibrated to a temperature of 37°C

by a circulating water pump. Excised human cadaver skin (approximately 200 micron thickness) is placed onto each chamber. After sealing with an O-ring, the exposed skin surface area has a diameter of 15 mm and the area is 1.77 cm^2. A total of 54 diffusion cells are used in the study. Nine different formulations are tested; each formulation is done in six replicates. Skin from the abdominal area (dorsal) from a single human donor is used for the study. Depending upon the experimental design, skin can also be tape-stripped to the "glistening" point (removal of stratum corneum) before starting the experiment.

Test Formulations: Test formulations provided by Dow Dermatologics, Inc., containing non-radioactive drug substance at the formulated concentration, are spiked with ^{14}C or ^{3}H-radiolabeled drug substance and mixed thoroughly with a spatula; this is done immediately prior to application to the skin chamber. The target for specific activity is about 0.050 µCi/mg test formulation. Spiked formulations are tested for uniform specific activity by liquid scintillation counting.

Test Formulation Application: Test formulations are applied to the skin surface with a Gilson positive displacement pipet. The amount applied is 17 mg/cm^2; therefore, about 30 mg of test formulation is applied to each cell.

Time Points: A sample of 1.0 mL is taken with a Gilson pipet from the reservoir under the skin sample at the following time points: 0, 1, and 24 hours. The sample is placed in a vial containing Ecoscint scintillation fluor. At the 0 and 1 hour time points the 1.0 mL volume is replaced with the BSA saline solution. The 0 and 1 hour time points are used to assess the integrity of the skin sample. If any holes or other defects exist, the 1 hour reservoir sample will show unusually high

radioactivity. In such cases that cell is removed from the study. It is common that several cells are omitted from the data calculations for each study. The endpoint can be varied from 3-24 hours. Additional time points can be added to the test protocol, at additional cost.

Washes and Gauze Swipes: At the end of the experiment, the skin is removed from the cell. To recover excess formulation from the skin surface, the skin is first washed with three 1.0 mL volumes of 2% Oleth-20 in water; this is retained for counting. The skin is then gently wiped with three separate cotton gauzes which are saved and Counted.

Skin Compartment Determinations: The skin is tape-stripped with cellophane tape until "glistening" (approximately 22 strips). The first two strips that remove the excess drug adhering to the outer surface of the stratum corneum (SC) are counted separately. These counts are included in total recovery but excluded from SC compartment recovery. Four groups each consisting of five consecutive tape strips are placed in a scintillation vial containing Scintilene. Dermis and epidermis are separated by microwave technique and placed in vials containing ReadyProt cocktail for tissue digestion. All samples are counted in a Beckman LSC counter and corrected for quenching. Recovery in the reservoir, washes, gauze wipes, and the respective skin compartments (SC, epidermis, and dermis) is calculated by determining the percent of the total counts applied. Total radioactive recovery is typically 70-100%.

Example 11: Process for Making 1% and 4% Al Ointment

(1) In a clean vessel, weigh micronized Al and Mineral Oil. Using a stainless steel spatula, spatulate the mixture until a homogeneous paste is achieved.

(2) In a separate vessel, weigh White Petrolatum and Glycerol Monostearate. Heat to approximately 75°C until melted.

(3) After mixing, add Oleic Acid and Butylated Hydroxytoluene to Step 2. Mix for approximately 5 minutes until homogeneous.

(4) With continuous mixing, add Benzyl Alcohol to Step 3. Mix for 5 minutes.

With continuous mixing, add Step 1 to Step 4. Mix for approximately 20 minutes to ensure Homogeneity.

Begin cooling process using a water bath, with continuous mixing and side scraping. Continue mixing and cooling until temperature is below 30°C.

VI. Synthesis of Quinazoline Derivatives

European Patent Application Publication No. 0 566 226 A1 (October 12, 1993) by Barker, A. J., incorporated by reference herein in its entirety, describes processes for the manufacture of certain quinazoline derivatives (e.g., pp 18-20 of EP 0 566 226 A1). However, conventional synthesis protocols for quinazoline derivatives have had relatively low yields and require reactions at high temperatures, the use of column chromatography and a 2-propanol solvent.

This invention features new and improved processes for the preparation of quinazolone, chloroquinazoline, 4-arylamino quinazoline, and their derivatives. Much better yields of the intermediate molecules and the final product are achieved with the processes of this invention than with conventional methods. The reaction to prepare quinazolone is conducted at a lower and more convenient temperature. In the isolation and purification of choloroquinazoline, column chromatography is not required. The final free base form of the 4-arylamino quinazoline is

obtained in high purity from the reaction mixture by simply adding an alkaline solution.

In addition to quinazolone, other substituted monocyclic, bicyclic, heterocyclic or polycyclic fused ring compounds and their salts containing a pyrimidone ring (see Figure 1 and below) can be prepared with the process of this invention from their β-amino acrylic acid precursors which contain a β-amino acrylic acid functionality such as acid, ester or -OC(O)-.

Thus, in one aspect, this invention features a process of preparing a substituted monocyclic, bicyclic, heterocyclic or polycyclic fused ring compound containing a pyrimidone ring by reacting a precursor which has a β-amino acrylic acid functionality with formamidine or a formamidine salt to fuse the pyrimidone ring. In preferred embodiments, the reaction is conducted with a formamidine salt in refluxing alcohol (e.g., ethanol). The reaction is conducted at a temperature of no less than 20°C (preferably no less than 60°C, and more preferably no less than 80°C) and no more than 150°C (preferably no more than 120°C, and more preferably no more than 100°C). The fused ring compound is quinazolone or a quinazolone derivative and the precursor is an aminobenzoate or amino benzoic acid. Specific compounds which can be prepared by this process are described in the detailed description of the invention.

In another aspect, this invention features a process of preparing a 4-halogen-pyrimidine (e.g. chloroquinazoline) by reacting a pyrimidone (e.g. quinazolone or a quinazolone derivative) with a halogenating agent (e.g. chlorinating agent) and isolating or purifying the end product, 4-halogen-pyrimidine (e.g. chloroquinazoline), by precipitation, crystallization or sublimation. In preferred embodiments, the pyrimidone

reacts with the halogenating agent in the presence of a catalyst such as dimethylformamide which promotes the formation of the 4-halogen-pyrimidine. The halogenating agent includes, but is not limited to, thionyl chloride, phosphorus oxychloride and oxalyl chloride. This process may be modified by replacing the halogenating agent with another displacement agent to prepare a quinazoline whose displaceable group is alkoxy, aryloxy or sulphonyloxy. For example, the displaceable group may be methoxy, phenoxy, methanesulphonyloxy or toluene-*p*-sulphonyloxy.

In yet another aspect, this invention features a process for preparing a 4-arylaminopyrimidine hydrochloride salt by dissolving a substitued aniline in an alcohol solution (e.g., ethanol), adding a chloropyrimidine to the alcohol solution to react with the substituted aniline, and isolating the end product, 4-arylaminopyrimidine hydrochloride salt. In a preferred embodiment, the 4-arylaminopyrimidine hydrochloride salt is a 4-arylamino quinazoline hydrochloride salt and the chloropyrimidine is chloroquinazoline or a chloroquinazoline derivative.

In addition, this invention features a process for preparing a free base 4-arylaminopyrimidine directly by dissolving a substitued aniline in an alcohol solution (e.g., ethanol), adding a chloropyrimidine to the alcohol solution to react with the substituted aniline, adding an alkaline solution to the alcohol solution, and isolating the end product, 4-arylaminopyrimidine. In a preferred embodiment, the 4-arylaminopyrimidine is a 4-arylamino quinazoline and the chloropyrimidine is chloroquinazoline or a chloroquinazoline derivative.

Furthermore, this invention features a process for converting an isolated 4-arylaminopyrimidine hydrochloride salt to its free base form by mixing it with an alkaline

solution and isolating the end product, 4-arylaminopyrimidine (e.g. 4-arylamino quinazoline).

Definition of Terms

As used hereinafter, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons, such as, e.g., methyl, ethyl, *n*-propyl, *iso*-propyl, *n*-butyl, *sec*-butyl, *iso*-butyl, *tert*-butyl, and 2-methylpentyl. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably hydroxyl, cyano, alkoxy, =O, =S, NO_2, $N(CH_3)_2$, amino, or SH.

An "alkoxy" group refers to an "-O-alkyl" group, where "alkyl" is defined as described above, such as methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, *n*-butoxy, *sec*-butoxy, *iso*-butoxy, *tert*-butoxy, trifluoromethoxy, 3-hydroxyhexyloxy, 2-carboxypropyloxy, 2-fluoroethoxy, carboxymethoxy and cyanobutyloxy and the like.

An "alkenyl" group refers to an unsaturated hydrocarbon group containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 2 to 7 carbons, more preferably 2 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably hydroxyl, cyano, alkoxy, =O, =S, No_s, halogen, $N(CH_2)_2$, amino, or SH. An "alkynyl" group refers to an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 2 to 12

carbons. More preferably, it is lower alkynyl of from 2 to 7 carbons, more preferably 2 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably hydroxyl, cyano, alkoxy, =O, =S, No_s, $N(CH_3)_2$, amino or SH.

A "heterocycle" denotes a chain of carbon and at least one non-carbon atoms which together form one or more aromatic or non-aromatic rings having preferrably between about 5-14 atoms, such as, e.g., furyl, thienyl, imidazolyl, indolyl, pyridinyl, thiadiazolyl, thiazolyl, piperazinyl, dibenzfuranyl, dibenzthienyl. These rings may be optionally substituted with one or more functional groups which are attached commonly to such rings, such as, e.g., hydroxyl, bromo, fluoro, chloro, iodo, mercapto or thio, cyano, cyanoamido, alkylthio, heterocycle, aryl, heteroaryl, carboxyl, oxo, alkoxycarbonyl, alkyl, alkenyl, nitro, amino, alkoxyl, amido, and the like to form rings such as, e.g., 2-aminothiazol-4-yl, 2-amino-5-chlorothiazol-4-yl, 2-amino-thiadiazol-4-yl, 2,3-dioxopiperazinyl, 4-alkylpiperazinyl, 2-iodo-3-dibenzfuranyl and 3-hydroxy-4-dibenzthienyl and the like.

An "aryl" group refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. Preferably, the aryl is a substituted or unsubstituted phenyl or pyridyl. Preferred aryl substituent(s) are halogen, trihalomethyl, hydroxyl, SH, OH, NO_2, amine, thioether, cyano, alkoxy, alkyl, and amino groups.

An "alkylaryl" group refers to an alkyl as described above covalently joined to an aryl group as described above. Preferably, the alkyl is a lower alkyl.

"Carbocyclic aryl" groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted.

"Heterocyclic aryl" groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen. The heterocyclic aryl groups of this invention include, but are not limited to, furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted.

An "aryloxy" denotes -OAr, where Ar is an aryl group as defined above.

An "aralkyl" denotes -RAr, where R is alkyl and Ar is aryl, both as defined above.

An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen.

An "amine" refers to a -N(R")R"', where R" and R"', is independently either hydrogen, alkyl, aryl, or alkylaryl, provided that R" and R"' are not both hydrogen.

An "amino" denotes the group NRR', where R and R' may independently be alkyl, aryl or acyl as defined above, or hydrogen.

A "cyanoamido" refers to the group -NH-C≡N.

General Formulas

Compounds of the formulas as depicted in Figures 1a-1d, 2a-2d, and their hydrochloride salts can be prepared by the process of this invention.

In Figures 1a, 1b, 1c and 1d:

X = 0, 1 or 2.

R1 is either hydrogen, hydroxy, halogen, trifluoromethyl, C1-C6 alkoxy, C1-C6 alkyl, nitro, cyano, or amino.

R2 and R3 are independently either hydrogen, C1-C6 alkyl, C1-C6 alkyl, C1-C6 alkoxy, cyclo (C1-C3 alkenedioxy), nitro, halogen, C1-C6 alkoxycarbonyl, cyano, or amido.

M and *N* are independently either single or double bonds. If *M* and *N* are single bonds then the bonds between atoms *B* and *C* can be a double bond.

Each of *A*, *B*, *C* and *D* is independently carbon or a heteroatom such as N, O, or S to give bicycles including, but not limited to, quinazoline derivatives. For example:

With both *M* and *N* double bonds

A	*B*	*C*	*D*	ring system
N	C	C	N	pteridine
C	N	N	C	pyrazino[2,3d]pyrimidine
N	C	C	C	pyridino[2,3-d]pyrimidine
C	N	C	C	pyridino[3,4-d]pyrimidine

With both *M* and *N* single bonds and the bond between β and γ a double bond

A	*B*	*C*	*D*	ring system
S	C	C	S	1,4-dithia-1,4-dihydroquinazoline
S	C	S	S	1,4-dithia-1,2,3,4-tetrahydroquinazoline

With both *M* and *N* single bonds

A	*B*	*C*	*D*	ring system
O	C	C	O	1,4-dioxolo[2,3-d]pyrimidine

In Figures 2a, 2b, 2c and 2d:

With *M* a single bond and *N* a double bond

A	*B*	*C*	ring system
S	C	C	thien[2,3-b]pyrimidine

O	C	C	furan[2,3-b]pyrimidine
N	C	C	pyrro[2,3-b]pyrimidine
C	C	C	cyclopenteno[2,3-b]pyrimidine
S	N	C	isothiazo[4,5-d]pyrimidine
S	C	N	thiazo[4,5-d]pyrimidine
O	N	C	isoxazo[4,5-d]pyrimidine
O	C	N	oxazo[4,5-d]pyrimidine

With *M* a double bond and *N* a single bond

A	*B*	*C*	Ring system
C	C	S	thien[2,3-b]pyrimidine
C	C	O	furan[2,3-b]pyrimidine
C	C	N	pyrro[2,3-b]pyrimidine
C	C	C	cyclopenten[3,4-b]pyrimidine
C	N	S	isothiazo[4,5-d]pyrimidine
N	C	S	thiazo[4,5-d]pyrimidine
C	N	O	isoxazo[4,5-d]pyrimidine
N	C	O	oxazo[4,5-d]pyrimidine

The process may also be employed for preparing substituted mono-, bi- and tricyclic fused ring compounds containing a pyrimidone ring by starting with any compound with a β-amino acrylic acid functionality. For example, the following tricyclic compounds can be prepared by this method: naptho[1,2-d]pyrimidine, naptho[2,3-d]pyrimidine, quinolino[2,3-d]pyrimidine, and quinolino[3,4-d]pyrimidine.

Quinazoline derivatives of formula V in Figure 4e and their pharmaceutically-acceptable salts and their precursors of formulae II and III may be prepared by the processes of this invention.

In formulas I, II, III and V, m is 1, 2 or 3 and n is 1 or 2. Each R^1 is independently selected from the group consisting of hydroxy, amino, carboxy, carbamoyl, ureido, (1-6C)alkoxycarbonyl, *N*-(1-6C)alkylcarbamoyl, *N,N*-di-[(1-6C)alkyl]carbamoyl, hydroxyamino, (1-6C)alkoxyami-

no, (2-6C)alkanoyloxyamino, trifluoromethoxy, (1-6C)alkyl, (1-6C)alkoxy, (1-3C)alkylenedioxy, (1-6C)alkylamino, di-[(1-6C)alkyl]amino, pyrrolidin-1-yl, piperidino, morpholino, piperazin-1-yl, 4-(1-6C)alkylpiperazin-1-yl, (1-6C)alkylthio, (1-6C)alkylsulphinyl, (1-6C)alkylsulphonyl, halogen-(1-6C)alkyl (other than trifluoromethyl), hydroxy-(1-6C)alkyl, (2-6C)alkanoyloxy-(1-6C)alkyl, (1-6C)alkoxy-(1-6C)alkyl, carboxy-(1-6C)alkyl, (1-6C)alkoxycarbonyl-(1-6C)alkyl, carbamoyl-(1-6C)alkyl, *N*-(1-6C)alkylcarbamoyl-(1-6C)alkyl, *N,N*-di-[(1-6C)alkyl]carbamoyl-(1-6C)alkyl, amino-(1-6C)alkyl, (1-6C)alkylamino-(1-6C)alkyl, di-[(1-6C)alkyl]amino-(1-6C)alkyl, piperidino-(1-6C)alkyl, morpholino-(1-6C)alkyl, piperazin-1-yl-(1-6C)alkyl, 4-(1-6C)alkylpiperazin-1-yl-(1-6C)alkyl, hydroxy-(2-6C)alkoxy-(1-6C)alkyl, (1-6C)alkoxy-(2-6C)alkoxy-(1-6C)alkyl, hydroxy-(2-6C)alkylamino-(1-6C)alkyl, (1-6C)alkoxy-(2-6C)alkylamino-(1-6C)alkyl, (1-6C)alkylthio-(1-6C)alkyl, hydroxy-(2-6C)alkylthio-(1-6C)alkyl, (1-6C)alkoxy-(2-6C)alkylthio-(1-6C)alkyl, phenoxy-(1-6C)alkyl, anilino-(1-6C)alkyl, phenylthio-(1-6C)alkyl, cyano-(1-6C)alkyl, halogen-(2-6C)alkoxy, hydroxy-(2-6C)alkoxy, (2-6C)alkanoyloxy-(2-6C)alkoxy, (1-6C)alkoxy-(2-6C)alkoxy, carboxy-(1-6C)alkoxy, (1-6C)alkoxycarbonyl-(1-6C)alkoxy, carbamoyl-(1-6C)alkoxy, *N*-(1-6C)alkylcarbamoyl-(1-6C)alkoxy, *N,N*-di-[(1-6C)alkyl]carbamoyl-(1-6C)alkoxy, amino-(2-6C)alkoxy, (1-6C)alkylamino-(2-6C)alkoxy, di-[(1-6C)alkyl]amino-(2-6C)alkoxy, (2-6C)alkanoyloxy, hydroxy-(2-6C)alkanoyloxy, (1-6C)alkoxy-(2-6C)alkanoyloxy, phenyl-(1-6C)alkoxy, phenoxy-(2-6C)alkoxy, anilino-(2-6C)alkoxy, phenylthio-(2-6C)alkoxy, piperidino-(2-6C)alkoxy, morpholino-(2-6C)alkoxy, piperazin-1-yl-(2-6C)alkoxy, 4-(1-6C)alkylpiperazin-1-yl-(2-6C)alkoxy, halogen-(2-6C)alkylamino, hydroxy-(2-6C)alkylamino, (2-

6C)alkanoyloxy-(2-6C)alkylamino, (1-6C)alkoxy-(2-6C)alkylamino, carboxy-(1-6C)alkylamino, (1-6C)alkoxycarbonyl-(1-6C)alkylamino, carbamoyl-(1-6C)alkylamino, *N*-(1-6C)alkylcarbamoyl-(1-6C)alkylamino, *N,N*-di-[(1-6C)alkyl]carbamoyl-(1-6C)alkylamino, amino-(2-6C)alkylamino, (1-6C)alkylamino-(2-6C)alkylamino, di-[(1-6C)alkyl]amino-(2-6C)alkylamino, phenyl-(1-6C)alkylamino, phenoxy-(2-6C)alkylamino, anilino-(2-6C)alkylamino, phenylthio-(2-6C)alkylamino, (2-6C)alkanoylamino, (1-6C)alkoxycarbonylamino, (1-6C)alkylsulphonylamino, benzamido, benzenesulphonamido, 3-phenylureido, 2-oxopyrrolidin-1-yl, 2,5-dioxopyrrolidin-1-yl, halogen-(2-6C)alkanoylamino, hydroxy-(2-6C)alkanoylamino, (1-6C)alkoxy-(2-6C)alkanoylamino, carboxy-(2-6C)alkanoylamino, (1-6C)alkoxycarbonyl-(2-6C)alkanoylamino, carbamoyl-(2-6C)alkanoylamino, *N*-(1-6C)alkylcarbamoyl-(2-6C)alkanoylamino, *N,N*-di-[(1-6C)alkyl]carbamoyl-(2-6C)alkanoylamino, amino-(2-6C)alkanoylamino, (1-6C)alkylamino-(2-6C)alkanoylamino and di-[(1-6C)alkyl]amino-(2-6C)alkanoylamino, and wherein said benzamido or benzenesulphonamido substituent or any anilino, phenoxy or phenyl group in a R^1 substituent may optionally bear one or two halogen, (1-6C)alkyl or (1-6C)alkoxy substituents. In addition, each R^2 of formula IV or V is independently selected from the group consisting of hydrogen, hydroxy, halogen, trifluoromethyl, amino, nitro, cyano, (1-6C)alkyl, (1-6C)alkoxy, cyclo[(1-3C)alkenedioxy], (1-6C)alkylamino, di-[(1-6C)alkyl]amino, (1-6C)alkylthio, (1-6C)alkylsulphinyl and (1-6C)alkylsulphonyl. Z of formula IV is a displaceable group which includes, but is not limited to, halogen, alkoxy, aryloxy and sulphonyloxy groups. Preferably, Z is selected from the group consisting of chloro, bromo,

methoxy, phenoxy, methanesulphonyloxy and toluene-*p*-sulphonyloxy groups.

In a preferred embodiment, each R^1 is independently selected from the group consisting of hydroxy, amino, ureido, methoxycarbonyl, ethoxycarbonyl, hydroxyamino, trifluoromethoxy, methyl, ethyl methoxy, ethoxy, propoxy, isopropoxy, butoxy, methylenedioxy, ethylenedioxy, methylamino, ethylamino, dimethylamino, diethylamino, piperidino, morpholino, mehtylthio, ethylthio, bromomethyl, dibromomethyl, methoxymethyl, piperidinomethyl, morpholinomethyl, piperazin-1-ylmethyl, methoxyethoxymethyl, methylthiomethyl, 2-hydroxyethylthiomethyl, anilinomethyl, phenylthiomethyl, cyanomethyl, 2-bromoethoxy, 2-hydroxyethoxy, 3-hydroxypropoxy, 2-methoxyethoxy, 2-ethoxyethoxy, 3-methoxypropoxy, 3-ethoxypropoxy, methoxycarbonylmethoxy, ethoxycarbonylmethoxy, carbamoylmethoxy, 2-dimethylaminoethoxy, 2-diethylaminoethoxy, 2-methoxyacetoxy, benzyloxy, 2-anilinoethoxy, 2-peperidinoethoxy, 2-morpholinoethoxy, 2-(piperazin-1-yl)ethoxy, 2-hydroxyethylamino, 3-hydroxypropylamino, 2-methoxyethylamino, 2-ethoxyethylamino, 3-methoxypropylamino, 3-ethoxypropylamino, 2-dimethylaminoethylamino, 2-diethylaminoethylamino, 3-dimethylaminopropylaminno, 3-diethylaminopropylamino, acetamido, propionamido, benzamido, 3-phenylureido, 2-chloroacetamido, 2-oxopyrrolidin-1-yl, 2-hydroxyacetamido, 2-methoxyacetamido and 2-ethoxyacetamido. In addition, each R^2 is independently selected from the group consisting of hydrogen, fluoro, chloro, bromo, trifluoromethyl, nitro, cyano, methyl and ethyl.

In a further preferred embodiment, $(R^1)_m$ is selected from the group consisting of 6-hydroxy, 7-hydroxy, 6,7-dihydroxy, 6-amino, 7-amino, 6-ureido, 6-trifluoromethoxy,

6-methyl, 6,7-dimethyl, 6-methoxy, 7-methoxy, 6,7-dimethoxy, 6,7-diethoxy, 6-hydroxy-7-methoxy, 7-hydroxy-6-methoxy, 6-amino-7-methoxy, 6-amino-7-methylthio, 5-amino-6,7-dimethoxy, 6-methoxy-7-isopropoxy, 6,7-methylenedioxy, 6,7-ethylenedioxy, 6-methylamino, 7-methylamino, 6-dimethyiamino, 6-amino-7-methylamino, 6-methoxymethyl, 6-bromomethyl, 6-(2-methoxyethoxymethyl), 6-cyanomethyl, 6-methylthiomethyl, 6-phenylthiomethyl, 7-(2-hydroxyethoxy)-6-methoxy, 6,7-di-(2-hydroxyethoxy), 6-(2-bromoethoxy), 6-(2-methoxyethoxy), 6-methoxy-7-(2-methoxyethoxy), 6,7-di-(2-methoxyethoxy), 7-(2-bromoethoxy)-6-methoxy, 7-benzyloxy-6-methoxy, 6-(2-methoxyethylamino), 6-acetamido, 6-benzamido, 6-(2-chloroacetamido), 6-(2-methoxyacetamido) and 7-(2-methoxyacetamido). In addition, $(R^2)_n$ is selected from the group consisting of hydrogen, 4'-fluoro, 3'-chloro, 3'-bromo, 3',4'-dichloro, 4'-fluoro-3'-chloro, 3'-trifluoromethyl, 4'-fluoro-3'-trifluoromethyl, 3'-nitro, 3'-nitro-4'-chloro, 3'-nitro-4'-flouro and 3'-methyl groups.

General synthesis process

A quinazoline derivative as depicted in Figures 1 and 2, or a pharmaceutically acceptable salt thereof, can be prepared by the processes shown in Figures 3a, 3b and 3c.

Step 1

An aspect of this invention is the improved yield and low reaction temperature in the first step accomplished by the use of formamidine or a formamidine salt instead of formamide. For example, the commercially available 6,7-dimethoxyanthranilic acid methyl ester is condensed with formamidine acetate by refluxing in ethanol to give 6,7-dimethoxyquinazolone in greater than 90%

yield. Similarly, 6,7-dimethoxyanthranilic acid is also condensed with formamidine to give >80% yield. This reaction is not restricted to unsubstituted formamidines but may employ substituted formamidines to give 3-substituted quinazolones.

This step converts a substituted ortho-aminobenzoic ester or a substituted isatoic anhydride or hydrochloride to give a quinazolone. The reaction is preferably carried out by heating in the presence of an inert solvent such as methanol, ethanol, isopropanol, a chlorinated solvent such as dichloromethane or chloroform, an ether such as tetrahydrofuran or dioxane, an amide such as dimethylformamide or dimethylacetamide, water or any other inert solvent. The reaction is heated to between 20°C and the boiling point of the solvent, preferably heating to between 20°C and 120°C. The quinazolone is obtained by cooling the reaction to ambient temperature or below and filtering the solid product, and, optionally, recrystallizing.

Alternatively, condensing with formamide at a temperature of about 170° C gives a yield of 75%, which is not as high as with a formamidine in ethanol and at a less convenient temperature.

Products which are not quinazoline or quinazoline derivatives but which contain heteroatom substituents in the benzene ring of the quinazoline and/or are saturated in this ring can also be prepared by this method. In Figures 3a-3c, Y is alkoxy or hydroxy.

Step 2

The compound prepared in Step 1 is reacted with an excess of thionyl chloride with a catalyst such as dimethylformamide in the absence of solvent or in the presence of a trapping agent such as toluene, benzene, or

xylene to remove free chlorine. Alternatively, another chlorinating agent can be used such as phosphorus oxychloride or oxalyl chloride. The crude product is isolated by evaporating the solvent and/or adding a co-solvent to precipitate it. The crude product, either as the solid or in solution in an inert solvent such as any of the solvents of Step 1, is washed with dilute aqueous base, such as sodium carbonate or bicarbonate and isolated by evaporation or precipitation with an inert co-solvent such as toluene, benzene or hexane. The washed product is satisfactory for conversion to the quinazoline in Step 3 or can be further purified by crystallization or sublimation.

For example, 6,7-dimethoxy-4-quinazolone can be reacted with thionyl chloride and dimethylformamide to form 4-chloro-6,7-dimethoxyquinazoline, which is purified by precitation and washing or sublimation. Such process requires no solvent and produces 4-chloro-6,7-dimethoxyquinazoline of high purity. Compared to conventional methods, this process has a much better yield (about 89% versus about 27%) and eliminates the chromatography step.

Step 3

The chloroquinazoline of Step 2 is reacted with a substituted aniline present in slight excess in the absence of solvent or in an inert solvent, or with an equivalent of aniline and a suitable base such as pyridine, triethylamine, ethyl morpholine, or any other unreactive base or an inorganic base such as sodium or potasium carbonate or bicarbonate. The inert solvent can be methanol, ethanol, isopropanol, a chlorinated solvent such as dichloromethane or chloroform, an ether such as tetrahydrofruan, 1,2-dimethoxyethane or dioxane, an amide

such as dimethylformamide or dimethylacetamide, or dimethylsulfoxide. The reaction is carried out between 20°C and the boiling point of the solvent, preferably between 20°C and 150°C. The solid product quinazoline hydrochloride salt is isolated by cooling and/or concentrating and filtering.

For example, 4-chloro-6,7-dimethoxyquinazoline is reacted with 3-chloroaniline to form 4-(3-chlorophenylamino)-6,7-dimethoxyquinazoline. In this invention, denatured alcohol was used as solvent instead of 2-propanol used in conventional methods. Applicant found that 4-chloro-6,7-dimethoxyquinazoline does not react with the alcoholic solvent as long as the aniline is already present in the mixture.

Step 4

An alkaline solution can be added directly to the reaction mixture of Step 3, stirred, and the free base isolated in Step 3 instead of the hydrochloride salt. This is simpler than conventional methods in which an additional step is required to obtain the free base quinazoline from its hydrochloride salt.

Alternatively, the quinazoline hydrochloride salt prepared in Step 3 is converted into its free base by treatment of the salt alone, or suspended in an inert organic solvent as described above, with a suitable organic or inorganic base as described above. The product is isolated by filtration with or without concentrating.

The following six compounds of formula V were prepared with the process of this invention.

Examples	R^1	R^2
S1	6,7-dimethoxy	3-chloro
S2	6,7-dimethoxy	3-bromo
S3	6,7-dimethoxy	3-trifluoromethyl
S4	6,7-dimethoxy	3-cyano
S5	6-methyl	3-chloro
S6	6-methyl	3-trifluoromethyl

Example 12:

4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline

Step 1

660 g of methyl 2-amino-4,5-dimethoxybenzoate, 660 g of formamidine acetate, and 2.46 kg of ethanol were stirred and refluxed in a 22 L flask for 10 hours. Heating was discontinued and the reaction was allowed to cool. The product was collected by vacuum filtration and washed with warm ethanol. The product was dried at 55°C under vacuum to give 609 g (about 95%) of 6,7-dimethoxy-4-quinazolone.

Alternatively, formamide (73.71g) and 82.80 g of methyl 2-amino-4,5-dimethoxybenzoate were stirred and heated at 170°C in a 250 ml flask maintained in a nitrogen atmosphere for 5.7 hours. Heating was discontinued and the reaction was allowed to cool. The mixture was diluted with 150 ml of water and the solids collected by vacuum filtration and washed three times with 50 ml of water. The product was dried at 60°C under a vacuum of 0.1 mm Hg to give 59.0 g (about 73%) of 6,7-dimethoxy-4-quinazolone.

Step 2

Thionyl chloride (256.8 g), 59.0 g of 6,7-dimethoxy-4-quinazolone, and 5 ml of dimethylformamide were stirred and heated at 93°C for 9.3 hours. Heating was discontinued, the mixture cooled to room temperature,

transferred to an evaporating flask using 100 ml of methylene chloride, and evaporated to dryness at 50°C. The resulting solid was stirred with 400 ml of saturated sodium bicarbonate, collected by vacuum filtration, and washed repeatedly with water (400 ml total). The product was dried at 50 - 60°C under a vacuum of 0.1 mm Hg to give 63.7 g (~100 % yield) of crude 4-chloro-6,7-dimethoxyquinazoline.

The crude was dissolved in 825 ml of boiling hexane, hot filtered and allowed to crystallize. The solids were collected, washed with 80 ml of hexane and air dried to give 66 g of product. The crude solid was sublimed at 150°C under a vacuum of < 0.1 mm Hg to give, in three crops, 55.7 g (about 89 % yield) of an off-white solid, mp (180.8°C softens) 185.0 -186.5°C. The proton NMR spectrum was consistent with the expected structure. TLC (5 % methanol in methylene chloride, silica gel): Rf 0.30.

Alternatively, 4-chloro-6,7 -dimethoxyquinazoline can be prepared with the method described in Step 2 of Example 13 below.

Steps 3 and 4

To a 500 ml reaction flask was added 18.09 g of 3-chloroaniline, 150 ml of denatured alcohol and then 13.0 g of 4-chloro-6,7-dimethoxyquinazoline. The reaction was heated to 80 ± 5°C and stirred vigorously for one hour. The reaction was allowed to cool to room temperature to give a precipitate of 4-(3-chlorophenylamino)-6,7-dimethoxyquinazoline hydrochloride salt. The flask was then cooled to 0 - 4°C with stirring.

One Normal sodium hydroxide (58 ml, one equivalent) was added and the reaction stirred at 0 - 4°C for 30 minutes. The product was collected by vacuum filtration,

washing twice with 20 ml portions of ethanol/water 2/1 (v/v). The product was dried under a vacuum of 0.1 mm Hg at ambient temperature to give 16.7 g of 4-(3-chlorophenylamino)-6,7-dimethoxyquinazoline (about 84% yield). M.P. 178 - 181°C.

In order to prepare the hydrochloride salt of 4-(3-chlorophenylamino)-6,7-dimethoxyquinazoline, the reaction was conducted as above but omitting the addition of 1 N sodium hydroxide to the cooled reaction mixture. Instead, the cooled reaction mixture was stirred for 30 minutes at 0 - 4°C, the product collected by vacuum filtration, and washed twice with 20 ml portions of ethanol. The product was dried under a vacuum of 0.1 mm Hg at ambient temperature to give 4-(3-chlorophenylamino)-6,7-dimethoxyquinazoline hydrochloride salt.

Example 13:

4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline

Step 1

2.500 kg of 2-amino-4,5-dimethoxybenzoate and 1.48 kg of formamidine acetate in 9.300 kg of ethanol were refluxed for 10 hours (at which time thin layer chromatography showed completion of the reaction). The mixture was cooled to room temperature and the crude product isolated by vacuum filtration. The crude product was resuspended in 5.75 kg of ethanol and stirred at 40° C for 30 minutes. The mixture was cooled to room temperature, the product isolated by vacuum filtration and dried under vacuum at 55° C until the loss on drying was <1%. The yield was 2.351 kg (96.8%) of 6,7-dimethoxyquinazolone at a purity of 99.48% by HPLC relative area %.

Step 2

1.18 kg of 6,7 -dimethoxyquinazolone, 5.13 kg of thionyl chloride, 0.53 kg of toluene, and 0.094 kg of dimethylformamide were refluxed for 6 hours. Thin layer chromatography showed completion of the reaction. The mixture was cooled to room temperature and then 6.82 kg of toluene was added. The mixture was stirred for 30 minutes and the crude solid product colleted by vacuum filtration. The solid was suspended in 26.2 kg of methylene chloride and agitated at room temperature for about 1 hour with 8.24 kg of aqueous solution containing 0.37 kg of sodium carbonate. The organic layer was separated, dried with 0.59 kg of anhydrous sodium sulfate, filtered to remove the sodium sulfate, and concentrated at $\leq$40° C to about 25% of its original weight.

To the thick slurry of product in methylene chloride was added 11.15 kg of toluene. The mixture was again concentrated until its temperature reached about 80° C. The mixture was cooled to room temperature and the solid product was collected by vacuum filtration and dried under vacuum at 55° C to give 0.884 kg of 4-chloro-6,7 - dimethoxyquinazoline (68.7% yield), 98.87 % pure by HPLC area %. The second batch of 4-chloro-6,7 dimethoxyquinazoline was manufactured by the same process as the first batch to give 1.088 kg of 4-chloro-6,7 - dimethoxyquinazoline (85.7% yield), 99.32% pure by HPLC area %.

Step 3

To a solution of 0.734 kg of 3-bromoaniline in 6.9 kg of ethanol was added 0.874 kg of 4-chloro-6,7 - dimethoxyquinazoline. This mixture was refluxed for about 2 hours at which time thin layer chromatography showed completion of the reaction. The mixture was cooled to

room temperature and the solid product isolated by vacuum filtration. The product was washed by suspending in 6.1 kg of ethanol and refluxing for about 30 minutes. The mixture was cooled to room temperature and the solid product again collected by vacuum filtration to give 1.855 kg of 4-(3-bromophenol) amino-6,7 -dimethoxyquinazoline hydrochloride sale (93.7%) yield), 98.37% pure by HPLC area %. Batch of 4-(3-bromophenol) amino-6,7 -dimethoxyquinazoline hydrochloride was manufactured by the same process as the first batch to give 2.610 kg of crude 4-(3-bromophenol) amino-6.7 -dimethoxyquinazoline hydrochloride (104.6% yield), 99.04% pure by HPLC area %.

Step 4

4.400 kg of crude 4-(3-bromophenol amino-6,7 -dimethoxyquinazoline hydrochloride and 8.5 kg of water containing 0.34 kg of sodium hydroxide was stirred in 13.33 kg of ethanol at room temperature for about 90 minutes. The solid product was isolated by vacuum filtration, washed by resuspending in 13.6 kg of water and again collected by vacuum filtration. The solid product was washed again by suspending in 13.6 kg of ethyl acetate, refluxed for about one hour, and collected by vacuum filtration. Finally the product was washed by suspending in 13.6 kg of water, stirred about 30 minutes at 45° C, cooled to room temperature, and collected by vacuum filtration. Drying under vacuum at 55° C gave 2.689 kg of 4-(3-bromophenol) amino-6,7 -dimethoxyquinazoline (83% yield), 99.5% pure by HPLC relative area % and containing 4.57 % water.

Example 14:

6,7-Dimethoxy-4-[3-(trifluoromethyl)phenylamino]quinazoline

This compound was prepared from the 4-chloro-6,7-dimethoxyquinazoline of Example 12 and 3-(trifluoromethyl) aniline by the method of Example 12.

Example 15:

4-(3-Cyanophenylamino)-6,7-dimethoxyquinazoline

This compound was prepared from the 4-chloro-6,7-dimethoxyquinazoline of Example 12 and 3-cyanoaniline by the method of Example 12.

Example 16:

4-(3-Chlorophenylamino)-6-methylquinazoline

2-Amino-5-methyl benzoic and formamide were condensed as in Example 12 to give 6-methyl-4-quinazolone in about 70% yield. 6-Methyl-4-quinazolone was chlorinated mith thionyl chloride as in Example 12 to give 4-chloro-6-methylquinazoline in about 84% yield and purified by sublimation. 4-(3-Chlorophenylamino)-6-methylquinazoline was prepared from 4-chloro-6-methylquinazoline and 3-chloroaniline as in Example 12 in about 60% yield.

Example 17:

6-Methyl-4-[3-(trifluoromethyl)phenylamino]quinazoline

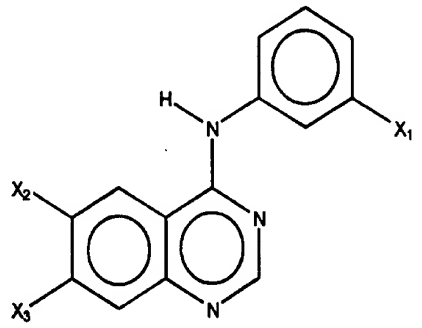
This compound was prepared from the 4-chloro-6-methylquinazoline of Example 13.

All publications referenced are incorporated by reference herein, including the nucleic acid sequences and amino acid sequences listed in each publication. All the compounds disclosed and referred to in the publications mentioned above are incorporated by reference herein,

including those compounds disclosed and referred to in articles cited by the publications mentioned above.

Other embodiments of this invention are disclosed in the following claims.

Table 1: Structures of Quinazoline Compounds.



Compound	X_1	X_2	X_3
A1	Br	methoxy	methoxy
A2	Cl	methoxy	methoxy
A3	Cl	methyl	H
A4	CF_3	methoxy	methoxy
A5	CN	methoxy	methoxy
A6	CF_3	methyl	H

Table 2: Inhibition of EGF-R Tyrosine Kinase Activityand Cellular Growth by Quinazoline derivatives

Compound	EGFR Kinase IC50 (μM)	3T3-EGFR IC50 (μM)	3T3-PDGFR IC50 (μM)	PDGFR/EGFR IC50/IC50
A4 free base	<0.006	0.4	28	70
A1 free base	0.0009	0.075	15	200
A2 free base	<0.015	0.11	18	163
A6 free base	0.9	6.0	30	5
A3 free base	0.058	2.0	10	5
A5 free base	0.2	1.5	>100	67

Table 3: Toxicity of Quinazoline Compounds (mg/kg)

	Toxicity* (mg/kg)		Plasma Concentration** (µg/mL)		
	LD_{10}	LD_{50}	5 min	15 min	60 min
A3	LD_{20}=400	>400	7.87±0.98	3.43±0.82	1.34±0.11
A2	>200 no deaths	>200 no deaths	11.5±4.0	6.5±1.3	3.7±0.5
A6	>400 no deaths	>400 no deaths	4.45±1.38	2.01±0.53	0.54±0.2
A1	75	~300	17.9±4.3	6.3±3.7	2.0±0.9
A4	LD_{40}=400	LD_{40}=400	13.3±1.5	12.7±3.0	3.9±1.5
A5	>153 no deaths	>153 no deaths	0.95±0.42	0.65±0.19	0.13±0.06

* Single dose, IP, in BALB/c, female mice.

** Single dose (50 mg/kg), IP, in BALB/c, nu/nu, female mice.

Table 4. EXCIPIENT SCREENING FOR A1

Excipient	Excipient Ratio	[Drug] (mg/mL)	Solubility After 24 Hrs.
Distilled H_2O			Insoluble
1.0N HCl			Insoluble/Yellow
Conc. NaOH			discoloration
			Insoluble
Ethanol			Partially Soluble
Polysorbate-80		10	Slightly Soluble
EtOH/Poly-80	50:50	10	Slightly Soluble
Benzyl Alcohol			Soluble
Triacetin			Partially Soluble
Oleic Acid			Insoluble/Yellow/Cloudy
Isopropyl Myristate			Insoluble/Cloudy
PEG-300			Soluble
Propylene Glycol			Slightly
Propylene			Soluble/Clear/Particulates
Carbonate			Soluble/80C/10 min.

Table 5: SOLUBILITY OF A1 IN PURE SOLVENTS

Compendial Excipients	Soluble at (% w/w)	Not Soluble at (% w/w)
Benzyl Alcohol, NF	3.60	4.77
Betizyl Berizoate, USP		0.85
Dehydrated Alcohol, USP	0.76	0.98
Isopropyl Alcohol, 99%, usp	_	1.25
Octyldodecanol, NF		0.68
Oleic Acid, NF		1.00
Polyethylene Glycol 300, USP	1.00	2.10
Polyethylene Glycol 400, USP	1.76	2.44
Polysorbate 20, NF		0.99
Propylene Carbonate, NF	0.98	1.46
Propylene Glycol, USP		0.59
Triacetin, USP		0.96
Non-Compendial Excipients		
Caprylic/Capric Triglyceride (Miglyol 812)		0.84
Diethyl Sebacate not compatible (yellow)		
Diisopropyl Adipate		0.66
Dimethyl isosorbide		0.97
Ethoxydiglycol (Transcutol)	2.41	3.74
Finsolve TN (Finetex)		1.27
Hexylene Glycol	0.98	2.05
Isotearyl Alcohol		0.99
Laureth 4	4.10	-

Table 6: A1 FORMULATIONS FOR SKIN PENETRATION STUDIES

Formulation Category	Drug Form	Major Excipients
1. Aqueous Gels	Solution	Water, PEG 400, Transcutol, propylene glycol, benzyl alcohol
2. Petrolatum	Solution	Petrolatum, Ointment propylene carbonate
3. PEG Ointments	Solution	PEG 400, benzyl y alcohol, Laureth-4, Transcutol
4. Creams	Suspended	Aqueous phase: propylene glycol, water, surfactants, Oil phase: mineral oil, stearyl alcohol.

Drug Concentration: 2% w/w in all the formulations (except aqueous gels)

Table 7: SUMMARY RESULTS A1 PENETRATION STUDY

	Polyethylene Glycol Ointments			
	SG-94A	SG-89A	SG-90A	SG-91A
Excipients	A	B	C	D
Polyethylene Glycol 400, NF	71.0	64.0	64.0	58.5
Benzyl Alcohol, NF	3.0	*****	*****	2.0
Laureth 4	*****	*****	*****	2.5
White Petrolatum, USP	*****	*****	*****	*****
Propylene Carbonate, NF	*****	*****	10.0	*****
Propylene Glycol, USP	*****	10.0	*****	10.0
Polyethylene Glycol 3350, NF	24.0	24.0	24.0	25.0
A1	2.0	2.0	2.0	2.0
Penetration Results				
μg Penetrated (R+D+E)*	1.8	1.6	1.9	3.2
μg Penetrated (R+D+E+SC)**	3.0	2.7	2.9	6.5
Percent of Dose (R+D+E)	0.3	0.3	0.3	0.5

	Petrolatum Ointments	
	SG-92A	SG-93A
Excipients	E	F
White Petrolatum, USP	83.5	82.5
Benzyl Alcohol, NF	1.5	*****
Lt. Mineral Oil, NF	5.0	*****
White Wax, NF	*****	5.0
Propylene Carbonate, NF	*****	7.5
White Wax, NF	*****	5.0
Glyceryl Monostearate, NF	3.0	3.0
A1	2.0	2.0
Oleic Acid, NF	5.0	*****
Penetration Results		
μg Penetrated (R+D+E)*	34.4	21.3
μg Penetrated (R+D+E+SC)**	47.2	33.6
Percent of Dose (R+D+E)	5.7	3.6

Emollient Creams

Excipients	SG-98A G	SG-98B H	SG-99A I
Stearyl Alcohol, USP	10.0	10.0	6.0
Cetyl Alcohol, USP	*****	*****	0.5
White Petrolatum, USP	5.0	5.0	*****
Octyldodecanol, NF	5.0	5.0	5.0
Sorbitan Monostearate, NF	*****	*****	1.0
Polyoxyl 40 Stearate, NF	*****	*****	4.0
Brij 721	2.0	2.0	*****
Brij 72	2.4	2.4	*****
Purified Water, USP	58.1	66.1	60.8
Propylene Glycol, USP	5.0	5.0	20.0
Benzyl Alcohol, NF	*****	2.0	*****
Ethoxydiglycol [Transcutol]	10.0	*****	*****
Methylparaben, USP	0.2	0.2	0.2
10% NaOH Solution	*****	*****	0.2
Carbopol 980, NF	*****	*****	0.3
Hydroxyethyl Cellulose 250HHX	0.3	0.3	*****
A1	2.0	2.0	2.0
Penetration Results			
μg Penetrated (R+D+E)*	16.5	20.7	14.8
μg Penetrated (R+D+E+SC)**	22.7	27.1	21.0
Percent of Dose (R+D+E)	2.8	3.4	2.5

*R+D+E = Reservoir + Dermis + Epidermis

**R+D+E+SC = Reservoir + Dermis = Epidermis + Stratum Corneum

Table 8. SKIN PENETRATION STUDIES

Formulation Category	No. of Formulations	Drug Form (2% w/w)	Major Excipients	Percent Skin Penetration*
Petrolatum Ointment	2	Suspended	White Petrolatum (84%), oleic acid, propylene carb., benzyl alcohol	3.6 - 5.7
Creams	3	Suspended	Water (60%), surfactant, alcohol propylene carb., mineral oil	2.5 - 3.4
Polyethylene Glycol Ointment	4	Dissolved	PEG -400 (58 - 71%) benzyl alcohol, laureth-4, transcutol	0.3 - 0.5

Table 9: A1 TOPICAL FORMULATION

FORMULATION DESIGNATION	PERCENT CONC. (w/w)
A1	1.0 or 4.0%
Mineral Oil, NF	5.00%
Glyceryl Monostearate, NF	3.00%
Benzyl Alcohol, NF	0.75%
Oleic Acid, NF	2.50%
Butylated Hydroxytoluene	0.001%
White Petrolatum, USP	QS to 100%

Table 10: Selectivity of A1

RECEPTOR	LIGAND	IC_{50} (μM)
EGF-R	EGF	0.02
EGF-R/Her-2	EGF	0.60
PDGF-bR	PDGF	>100
IGF-1R	IGF-1	>100
IR	Insulin	>100

Table 11: Effect of A1 on cell growth

CELL LINE	RTK	IC_{50} (μM)
A431	EGF-R	1.6
BT474	Her-2	0.9
C6	PDGF-bR	>100

Table 12. Skin penetration studies: recovery of A1 in the epidermis

Percent A1	Epidermis μg Recovery	Epidermis μM Concentration
0.5	1.6 ± 0.7	248 ± 107
1.0	2.3 ± 1.0	355 ± 151
2.0	4.5 ± 1.0	712 ± 157
4.0	16.3 ± 9.4	2561 ± 1469

Table 13: TOPICAL FORMULATION SERIES I

FORMULATION COMPONENTS	F1	F2
A1	2.0	2.0
White Petrolatum, USP	83.5	82.5
Benzyl Alcohol, NF	1.5	N/A
Light Mineral Oil, NF	5.0	N/A
Propylene Carbonate	N/A	7.5
White Wax, NF	N/A	5.0
Glyceryl Monostearate	3.0	3.0
Oleic Acid, NF	5.0	N/A
µg Penetrated (R+D+E+Sc)	47	34
% Penetrated (R+D+E+Sc)	7.9	5.6

Table 14: TOPICAL FORMULATION SERIES II

FORMULATION COMPONENTS	F1	F3	F4	F5	F6
Al	2.0	2.0	2.0	2.0	2.0
White Petrolatum, USP	83.5	81.8	80.3	85.8	78.3
Benzyl Alcohol, NF	1.5	3.0	1.5	1.5	1.5
Mineral Oil, USP	5.0	5.0	5.0	5.0	5.0
Propylene Glycol, USP	N/A	N/A	N/A	N/A	5.0
Laureth 4	N/A	N/A	3.0	N/A	N/A
Glyceryl Monostearate	3.0	3.0	3.0	3.0	3.0
Oleic Acid, NF	5.0	5.0	5.0	2.5	5.0
Vitamin E, USP	N/A	0.2	0.2	0.2	0.2
µg Penetrated (R+D+E+Sc)	44	28	22	36	9
% Penetrated (R+D+E+Sc)	7.4	4.6	3.6	6.1	1.4

Table 15: Topical Formulation Series III

PETROLATUM OINTMENTS

	F7	F8	F9	F10	F11	F12	F13	F14
WHITE PETROLATUM, USP	87.00	84.00	87.50	86.00	87.25	88.50	86.75	87.50
BENZYL ALCOHOL, NF	1.50	1.50	1.50	1.50	1.50	1.50	0.75	.....
MINERAL OIL, USP	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
GLYCERYL MONOSTEARATE	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
OLEIC ACID, NF	2.50	2.50	2.50	2.50	1.25	....	2.50	2.50
SU5271	1.00	4.00	0.50	2.00	2.00	2.00	2.00	2.00

Penetration Results

µg Penetrated (R+D+E+SC)**	21	81	12	38	41	31	44	38
Percent of Dose (R+D+E+SC)	7	6.7	7.8	5.3	6.9	5.2	7.3	6.4

Table 16: EXEMPLARY TOPICAL FORMULATION

FORMULATION DESIGNATION	PERCENT CONC. (w/w)
A1	0.25 or 1.0%
Mi	5.00%
Glyceryl Monostearate, NF	3.00%
Benzyl Alcohol, NF	0 to 1.50%
Oleic Acid, NF	2.50%
Butylated Hydroxytoluene, NF	0.001%
White Petrolatum, USP	QS to 100%

Claims

1. A method for treating a hyperproliferative skin disorder in a host, comprising the step of administering to said host a composition containing a pharmaceutically effective amount of a compound selected from the group consisting of 4-(3-Bromophenyl-amino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenyl-amino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenyl-amino)-6-methylquinazoline, 4-[3-(trifluoromethyl) phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyano-phenylamino)-6,7-dimethoxyquinazoline, 4-[3-(trifluoro-methyl)phenylamino]-6-methylquinazoline, and a pharmaceutically acceptable salt thereof.

2. The method according to claim 1 wherein said composition is administered to said host by topical application.

3. The method according to claim 1 wherein said hyperproliferative skin disorder is selected from the group consisting of psoriasis, hyperproliferation caused by papilloma virus infection, and skin cancer.

4. The method according to claim 1 wherein said compound is 4-(3-Bromophenylamino)-6,7-dimethoxy-quinazoline or a pharmaceutically acceptable salt thereof.

5. The method according to claim 1 wherein said composition comprises a pharmaceutically acceptable carrier.

6. A method for reducing keratinocyte proliferation in a host, comprising the step of

administering to said host a composition containing a pharmaceutically effective amount of a compound selected from the group consisting of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6-methylquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyanophenylamino)-6,7-dimethoxyquinazoline,4-[3-(trifluoromethyl) phenylamino]-6-methylquinazoline, and a pharmaceutically acceptable salt thereof.

7. A method for reducing skin lesions caused by Papilloma virus infection in a host, comprising the step of administering to said host a composition containing a pharmaceutically effective amount of a compound selected from the group consisting of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6-methylquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyanophenylamino)-6,7-dimethoxyquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6-methylquinazoline, and a pharmaceutically acceptable salt thereof.

8. A method for treating psoriasis in a host, comprising the step of administering to said host a composition containing a pharmaceutically effective amount of a compound selected from the group consisting of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6-methylquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyanophenylamino)-

6,7-dimethoxyquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6-methylquinazoline, and a pharmaceutically acceptable salt thereof.

9. A method for treating skin cancer in a host, comprising the step of administering to said host a composition containing a pharmaceutically effective amount of a compound selected from the group consisting of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6-methylquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyanophenylamino)-6,7-dimethoxyquinazoline, 4-[3-(trifluoromethyl)-phenylamino]-6-methylquinazoline, and a pharmaceutically acceptable salt thereof.

10. A pharmaceutical composition for the treatment of a hyperproliferative skin disorder comprising

(a) a pharmaceutically effective amount of a compound selected from the group consisting of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6-methylquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyanophenylamino)-6,7-dimethoxyquinazoline, 4-[3-(trifluoromethyl)-phenylamino]-6-methylquinazoline, and a pharmaceutically acceptable salt thereof; and

(b) a pharmaceutically acceptable carrier.

11. The pharmaceutical composition of claim 10, wherein said carrier is for topical application to a host.

12. The pharmaceutical composition of claim 10 wherein said compound consists of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline or a pharmaceutically acceptable salt thereof.

13. A method of making a pharmaceutical composition for the treatment of a hyperproliferative skin disorder, comprising the steps of:

(a) providing a pharmaceutically effective amount of a compound selected from the group consisting of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6-methylquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyanophenylamino)-6,7-dimethoxyquinazoline, 4-[3-(trifluoromethyl)-phenylamino]-6-methylquinazoline, and a pharmaceutically acceptable salt thereof;

(b) providing a pharmaceutically acceptable carrier; and

(c) admixing said compound with said carrier.

14. The method according to claim 13 wherein said pharmaceutically acceptable carrier is suitable for topical application.

15. The method according to claim 13 wherein said hyperproliferative skin disorder is selected from the group consisting of psoriasis, hyperproliferation caused by papilloma virus infection, and skin cancer.

16. The method according to claim 13 wherein said compound is 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline or a pharmaceutically acceptable salt thereof.

17. A method of making a topical formulation, comprising the steps of:

(a) providing a nonpolar hydrocarbon mixture;

(b) providing a pharmaceutically effective amount of a compound selected from the group consisting of quinazoline, a quinazoline derivative, and a pharmaceutically acceptable salt thereof;

(c) admixing said compound with said nonpolar hydrocarbon mixture to disperse said compound in said mixture;

(d) providing an effective amount of an excipient which in combination with said nonpolar hydrocarbon mixture enhances the ability of said compound to penetrate through the skin; and

(e) admixing said excipient with said nonpolar hydrocarbon mixture;

wherein said steps (b) and (c) are performed either before or after said steps (d) and (e).

18. The method of claim 17, wherein said nonpolar hydrocarbon mixture is selected from the group consisting of a petrolatum ointment and mineral oil.

19. The method of claim 17, wherein said excipient is oleic acid or benzyl alcohol.

20. The method of claim 19, wherein the concentration of said oleic acid is from about 0.05% to about 5.0% by weight in said topical formulation.

21. The method of claim 17, further comprising the step of admixing an effective amount of an antioxidant with said nonpolar hydrocarbon mixture.

22. The method of claim 21, wherein said antioxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, α-tocopherol, ascorbic acid, and propyl gallate.

23. The method of claim 21, wherein said antioxidant is butylated hydroxytoluene with a concentration of from about 0.0001% to about 0.01% by weight in the topical formulation.

24. The method of claim 19, wherein the concentration of said benzyl alcohol is from about 0.05% to about 3.0% by weight in said topical formulation.

25. The method of claim 17, wherein said compound is dispersed in said nonpolar hydrocarbon mixture in particles and more than half of said particles have a diameter of no more than about 50 microns.

26. The method of claim 17, wherein said compound is selected from the group consisting of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6-methylquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyanophenylamino)-6,7-dimethoxyquinazoline, 4-[3-(trifluoromethyl)-phenylamino]-6-methylquinazoline, and a pharmaceutically acceptable salt thereof.

27. The method of claim 17, wherein said compound is 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline or a pharmaceutically acceptable salt thereof.

28. The method of claim 27, wherein the concentration of said compound is from about 0.1% to about 4.0% by weight in said topical formulation.

29. A composition for topical application, comprising:

(a) a nonpolar hydrocarbon mixture;

(b) a pharmaceutically effective amount of a compound selected from the group consisting of quinazoline, a quinazoline derivative, and a pharmaceutically acceptable salt thereof; and

(c) an excipient; wherein said compound is dispersed in said nonpolar hydrocarbon mixture, and said excipient in combination with said nonpolar hydrocarbon mixture enhance the ability of said compound to penetrate through the skin.

30. The composition of claim 29, wherein said nonpolar hydrocarbon mixture is selected from the group consisting of a petrolatum ointment and mineral oil.

31. The composition of claim 29, wherein said excipient is oleic acid or benzyl alcohol.

32. The composition of claim 31, wherein the concentration of said oleic acid is from about 0.05% to about 5.0% by weight.

33. The composition of claim 29, further comprising an effective concentration of an antioxidant.

34. The composition of claim 33, wherein said antioxidant is selected from the group consisting of butylated hydroxyanisole, butylated hydroxytoluene, α-tocopherol, ascorbic acid, and propyl gallate.

35. The composition of claim 33, wherein said antioxidant is butylated hydroxytoluene with a concentration of from about 0.0001% to about 0.01% by weight.

36. The composition of claim 31, wherein the concentration of said benzyl alcohol is from about 0.05% to about 3.0% by weight.

37. The composition of claim 29, wherein said compound is dispersed in said nonpolar hydrocarbon mixture in particles and more than half of said particles have a diameter of no more than about 50 microns.

38. The composition of claim 29, wherein said compound is selected from the group consisting of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6,7-dimethoxyquinazoline, 4-(3-Chlorophenylamino)-6-methylquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6,7-dimethoxyquinazoline, 4-(3-Cyanophenylamino)-6,7-dimethoxyquinazoline, 4-[3-(trifluoromethyl)phenylamino]-6-methylquinazoline, and a pharmaceutically acceptable salt thereof.

39. The composition of claim 38, wherein said compound is 4-(3-Bromophenylamino)-6, 7-dimeth-

oxyquinazoline or a pharmaceutically acceptable salt thereof.

40. The composition of claim 39, wherein the concentration of said compound is from about 0.1% to about 4.0% by weight.

41. A petrolatum ointment for topical treatment of psoriasis, comprising:

from about 0.1% to about 4.0% by weight of 4-(3-Bromophenylamino)-6,7-dimethoxyquinazoline or a pharmaceutically acceptable salt thereof,

from about 1.0% to about 5.0% by weight of oleic acid, and

from about 0.0001% to about 0.01% by weight of butylated hydroxytoluene.

42. A process for preparing a substituted monocyclic, heterocyclic or polycyclic fused ring compound containing a pyrimidone ring, comprising the step of reacting a β-amino acrylic acid precursor of said compound with formamidine or a formamidine salt under conditions suitable to fuse said pyrimidone ring.

43. The process of claim 42, wherein said precursor is reacted with formamidine or a formamidine salt in refluxing ethanol.

44. The process of claim 42, wherein said fused ring compound containing a pyrimidone ring is quinazolone or a quinazolone derivative, and said precursor is 2-aminobenzoate or 2-amino benzoic acid.

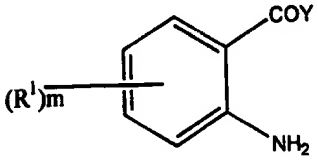
45. The process of claim 44, wherein the reaction is conducted at a temperature of no more than 150°C.

46. The process of claim 44, wherein the reaction is conducted at a temperature of no more than 120°C.

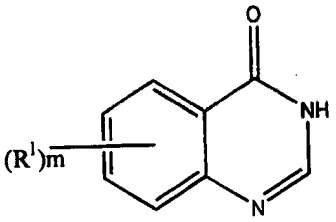
47. The process of claim 44, wherein the reaction is conducted at a temperature of no more than 100°C.

48. The process of claim 44, wherein said formamidine salt is formamidine acetate.

49. The process of claim 42, wherein said fused ring compound containing a pyrimidone ring has the structure of formula II and said precursor has the structure of formula I,



Formula I



Formula II

wherein Y represents hydroxy or an alkoxy group, m is 1, 2 or 3 and each R^1 is independently selected from the group consisting of hydroxy, amino, carboxy, carbamoyl, ureido, (1-6C)alkoxycarbonyl, *N*-(1-6C)alkylcarbamoyl, *N,N*-di-[(1-6C)alkyl]carbamoyl, hydroxyamino, (1-6C)alkoxyamino, (2-6C)alkanoyloxyamino, trifluoromethoxy, (1-6C)alkyl, (1-6C)alkoxy, (1-3C)alkylenedioxy, (1-6C)alkylamino, di-[(1-6C)alkyl]amino, pyrrolidin-1-yl, piperidino, morpholino, piperazin-1-yl, 4-(1-

6C)alkylpiperazin-1-yl, (1-6C)alkylthio, (1-6C) alkylsulphinyl, (1-6C)alkylsulphonyl, halogen-(1-6C)alkyl (other than trifluoromethyl), hydroxy-(1-6C)alkyl, (2-6C)alkanoyloxy-(1-6C)alkyl, (1-6C)alkoxy-(1-6C)alkyl, carboxy-(1-6C)alkyl, (1-6C)alkoxycarbonyl-(1-6C)alkyl, carbamoyl-(1-6C)alkyl, *N*-(1-6C) alkylcarbamoyl-(1-6C)alkyl, *N,N*-di-[(1-6C) alkyl] carbamoyl-(1-6C)alkyl, amino-(1-6C)alkyl, (1-6C)alkylamino-(1-6C)alkyI, di-[(1-6C)alkyl]amino-(1-6C)alkyl, piperidino-(1-6C)alkyl, morpholino-(1-6C)alkyl, piperazin-1-yl-(1-6C)alkyl, 4-(1-6C)alkylpiperazin-1-yl-(1-6C)alkyl, hydroxy-(2-6C)alkoxy-(1-6C)alkyl, (1-6C)alkoxy-(2-6C)alkoxy-(1--6C)alkyl, hydroxy-(2-6C)alkylamino-(1-6C)alkyl, (1-6C)alkoxy-(2-6C)alkylamino-(1-6C)alkyl, (1-6C)alkylthio-(1-6C)alkyl, hydroxy-(2-6C)alkylthio-(1-6C)alkyl, (1-6C)alkoxy-(2-6C)alkylthio-(1-6C)alkyl, phenoxy-(1-6C)alkyl, anilino-(1-6C)alkyl, phenylthio-(1-6C)alkyl, cyano-(1-6C)alkyl, halogen-(2-6C)alkoxy, hydroxy-(2-6C)alkoxy, (2-6C)alkanoyloxy-(2-6C)alkoxy, (1-6C)alkoxy-(2-6C)alkoxy, carboxy-(1-6C)alkoxy, (1-6C)alkoxycarbonyl-(1-6C)alkoxy, carbamoyl-(1-6C)alkoxy, *N*-(1-6C)alkylcarbamoyl-(1-6C)alkoxy, *N,N*-di-[(1-6C)alkyl]carbamoyl-(1-6C)alkoxy, amino-(2-6C)alkoxy, (1-6C)alkylamino-(2-6C)alkoxy, di-[(1-6C)alkyl]amino-(2-6C)alkoxy, (2-6C)alkanoyloxy, hydroxy-(2-6C)alkanoyloxy, (1-6C)alkoxy-(2-6C)alkanoyloxy, phenyl-(1-6C)alkoxy, phenoxy-(2-6C)alkoxy, anilino-(2-6C)alkoxy, phenylthio-(2-6C)alkoxy, piperidino-(2-6C)alkoxy, morpholino-(2-6C)alkoxy, piperazin-1-yl-(2-6C)alkoxy, 4-(1-6C)alkylpiperazin-1-yl-(2-6C)alkoxy, halogen-(2-6C)alkylamino, hydroxy-(2-6C)alkylamino, (2-6C)alkanoyloxy-(2-6C)alkylamino, (1-6C)alkoxy-(2-6C)alkylamino, carboxy-(1-6C)alkylamino, (1-

6C)alkoxycarbonyl-(1-6C)alkylamino, carbamoyl-(1-6C)alkylamino, *N*-(1-6C)alkylcarbamoyl-(1-6C)alkylamino, *N,N*-di-[(1-6C)alkyl]carbamoyl-(1-6C)alkylamino, amino-(2-6C)alkylamino, (1-6C)alkylamino-(2-6C)alkylamino, di-[(1-6C)alkyl]amino-(2-6C)alkylamino, phenyl-(1-6C)alkylamino, phenoxy-(2-6C)alkylamino, anilino-(2-6C)alkylamino, phenylthio-(2-6C)alkylamino, (2-6C)alkanoylamino, (1-6C)alkoxycarbonylamino, (1-6C)alkylsulphonylamino, benzamido, benzenesulphonamido, 3-phenylureido, 2-oxopyrrolidin-1-yl, 2,5-dioxopyrrolidin-1-yl, halogen-(2-6C)alkanoylamino, hydroxy-(2-6C)alkanoylamino, (1-6C)alkoxy-(2-6C)alkanoylamino, carboxy-(2-6C)alkanoylamino, (1-6C)alkoxycarbonyl-(2-6C)alkanoylamino, carbamoyl-(2-6C)alkanoylamino, *N*-(1-6C)alkylcarbamoyl-(2-6C)alkanoylamino, *N,N*-di-[(1-6C)alkyl]carbamoyl-(2-6C)alkanoylamino, amino-(2-6C)alkanoylamino, (1-6C)alkylamino-(2-6C)alkanoylamino and di-[(1-6C)alkyl]amino-(2-6C)alkanoylamino, and wherein said benzamido or benzenesulphonamido substituent or any anilino, phenoxy or phenyl group in a R^1 substituent may optionally bear one or two halogen, (1-6C)alkyl or (1-6C)alkoxy substituents.

50. The process of claim 49, wherein $(R^1)_m$ is selected from the group consisting of 6-hydroxy, 7-hydroxy, 6,7-dihydroxy, 6-amino, 7-amino, 6-ureido, 6-trifluoromethoxy, 6-methyl, 6,7-dimethyl, 6-methoxy, 7-methoxy, 6,7-dimethoxy, 6,7-diethoxy, 6-hydroxy-7-methoxy, 7-hydroxy-6-methoxy, 6-amino-7-methoxy, 6-amino-7-methylthio, 5-amino-6,7-dimethoxy, 6-methoxy-7-isopropoxy, 6,7-methylenedioxy, 6,7-ethylenedioxy, 6-methylamino, 7-methylamino, 6-dimethyiamino, 6-amino-7-methylamino, 6-methoxymethyl, 6-bromomethyl, 6-(2-

methoxyethoxymethyl), 6-cyanomethyl, 6-methylthiomethyl, 6-phenylthiomethyl, 7-(2-hydroxyethoxy)-6-methoxy, 6,7-di-(2-hydroxyethoxy), 6-(2-bromoethoxy), 6-(2-methoxyethoxy), 6-methoxy-7-(2-methoxyethoxy), 6,7-di-(2-methoxyethoxy), 7-(2-bromoethoxy)-6-methoxy, 7-benzyloxy-6-methoxy, 6-(2-methoxyethylamino), 6-acetamido, 6-benzamido, 6-(2-chloroacetamido), 6-(2-methoxyacetamido) and 7-(2-methoxyacetamido).

51. A process for preparing a 4-halogen-pyrimidine, comprising the steps of reacting pyrimidone or a pyrimidone derivative with a halogenating agent under conditions suitable for production of said 4-halogen-pyrimidine and isolating or purifying the end product by precipitation, crystallization or sublimation.

52. The process of claim 51, wherein said 4-halogen-pyrimidine is chloroquinazoline or a chloroquinazoline derivative, said pyrimidone derivative is quinazolone or a quinazolone derivative, and said halogenating agent is a chlorinating agent.

53. The process of claim 52, wherein said pyrimidone derivative is reacted with said chlorinating agent in the presence of a catalyst which promotes the formation of said chloroquinazoline or chloroquinazoline derivative and a trapping agent which removes free chlorine from the reaction.

54. The process of claim 53, wherein said catalyst is dimethylformamide and said trapping agent is toluene.

55. The process of claim 52, wherein said chlorinating agent is selected from the group consisting of thionyl chloride, phosphorus oxychloride and oxalyl chloride.

56. A process for preparing a 4-arylamino-pyrimidine hydrochloride salt, comprising the steps of dissolving a substituted aniline in an ethanol solution, adding to said solution a chloropyrimidine under conditions which cause reaction with said substituted aniline, and isolating the resulting 4-arylamino-pyrimidine hydrochloride salt from said solution.

57. The process of claim 56, wherein said 4-arylaminopyrimidine hydrochloride salt is a 4-arylamino quinazoline hydrochloride salt and said chloropyrimidine is chloroquinazoline or a chloroquinazoline derivative.

58. A process for preparing a 4-arylamino-pyrimidine, comprising the steps of dissolving a substituted aniline in ethanol, adding to said solution a chloropyrimidine to react with said substituted aniline, adding an alkaline solution to said ethanol solution, and isolating said 4-arylaminopyrimidine.

59. The process of claim 58, wherein said 4-arylaminopyrimidine is a 4-arylamino quinazoline and said chloropyrimidine is chloroquinazoline or a chloroquinazoline derivative.

60. A process for preparing a 4-arylamino-pyrimidine, comprising the steps of dissolving a substituted aniline in an ethanol solution, adding to said solution a chloropyrimidine under conditions which

cause reaction with said substituted aniline, isolating the resulting 4-arylaminopyrimidine hydrochloride salt from said solution, dissolving said 4-arylamino quinazoline hydrochloride salt in an alkaline solution, and isolating the resulting 4-arylaminopyrimidine.

61. The process of claim 60, wherein said 4-arylaminopyrimidine is a 4-arylamino quinazoline, said 4-arylaminopyrimidine hydrochloride salt is a 4-arylamino quinazoline hydrochloride salt, and said chloropyrimidine is chloroquinazoline or a chloroquinazoline derivative.

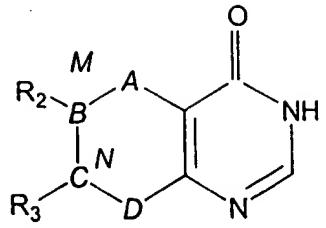


Fig. 1a

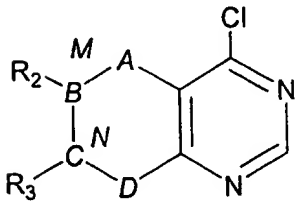


Fig. 1b

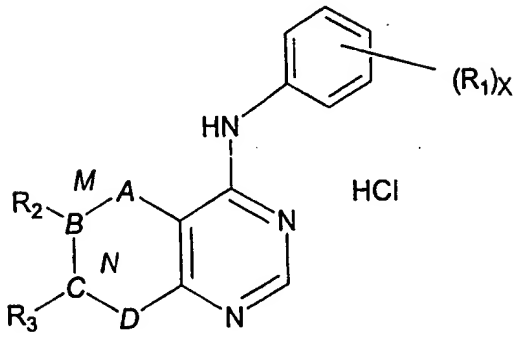


Fig. 1c

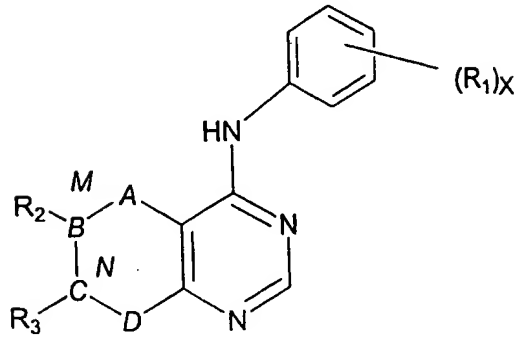


Fig. 1d

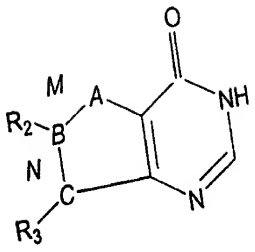


Fig. 2a

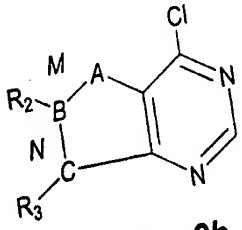


Fig. 2b

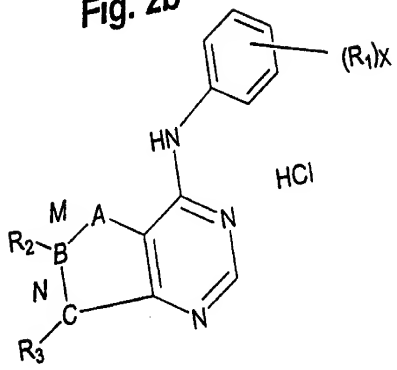


Fig. 2c

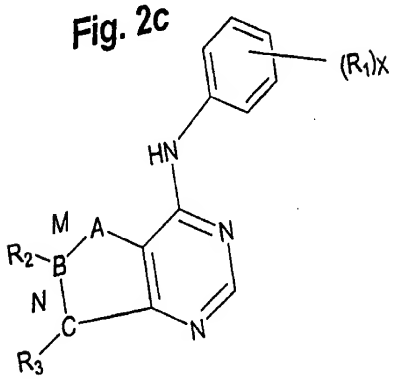


Fig. 2d

AMINOBENZOATE
FORMAMIDINE SALT/
SOLVENT, HEAT
STEP 1
QUINAZOLONE
CHLORINATING AGENT/SOCl2
CATALYST, HEAT
STEP 2
PURIFIED BY PRECIPITATION,
CRYSTALLIZATION OR SUBLIMATION
CHLOROQUINAZOLINE
SUBSTITUTE ANILINE/
SOLVENT, HEAT
STEP 3
QUINAZOLINE HYDROCHLORIDE SALT
SOLVENT, BASE
STEP 4
QUINAZOLINE

Fig. 3a

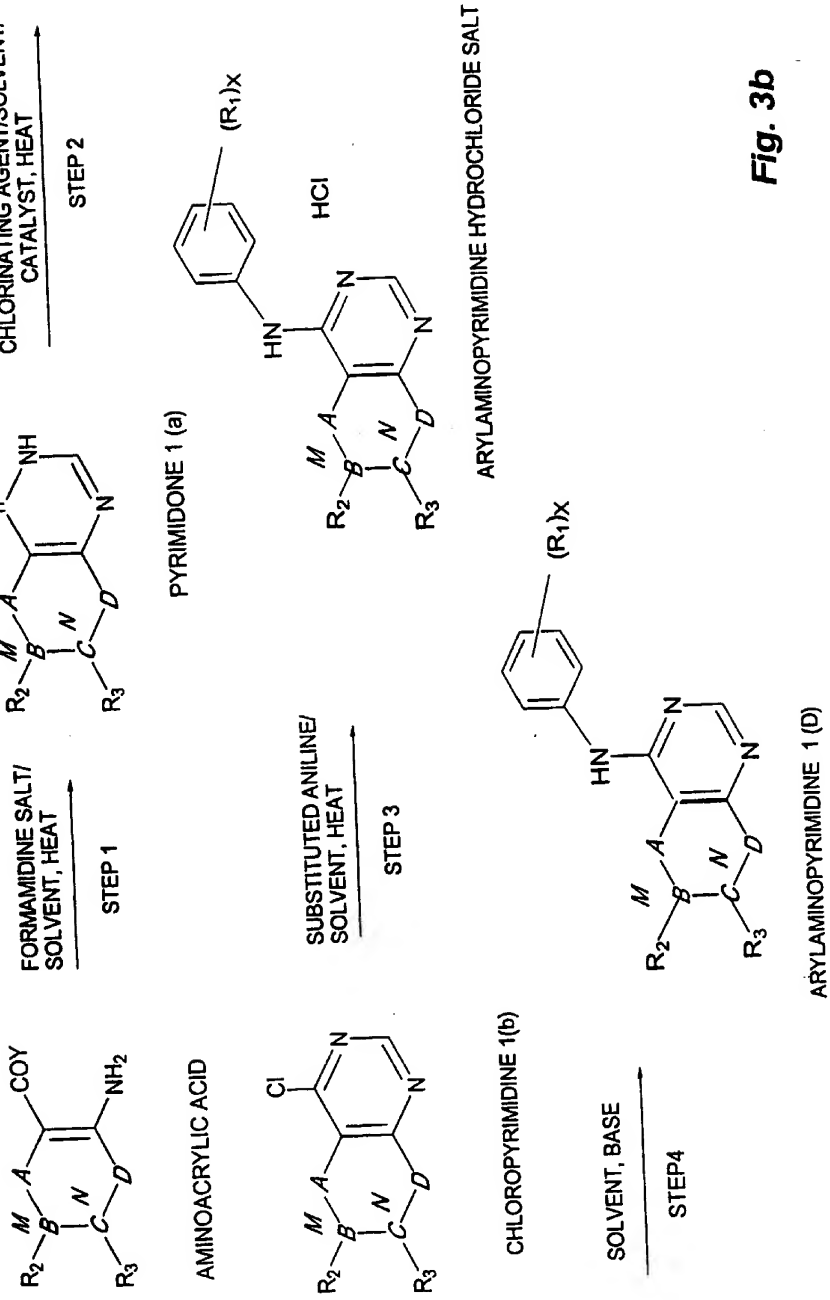

FORMAMIDINE SALT/ SOLVENT, HEAT
STEP 1
AMINOACRYLIC ACID
CHLORINATING AGENT/SOLVENT/ CATALYST, HEAT
STEP 2
PYRIMIDONE 1 (a)
SUBSTITUTED ANILINE/ SOLVENT, HEAT
STEP 3
ARYLAMINOPYRIMIDINE HYDROCHLORIDE SALT 1 (C)
CHLOROPYRIMIDINE 1(b)
SOLVENT, BASE
STEP4
ARYLAMINOPYRIMIDINE 1 (D)

Fig. 3b

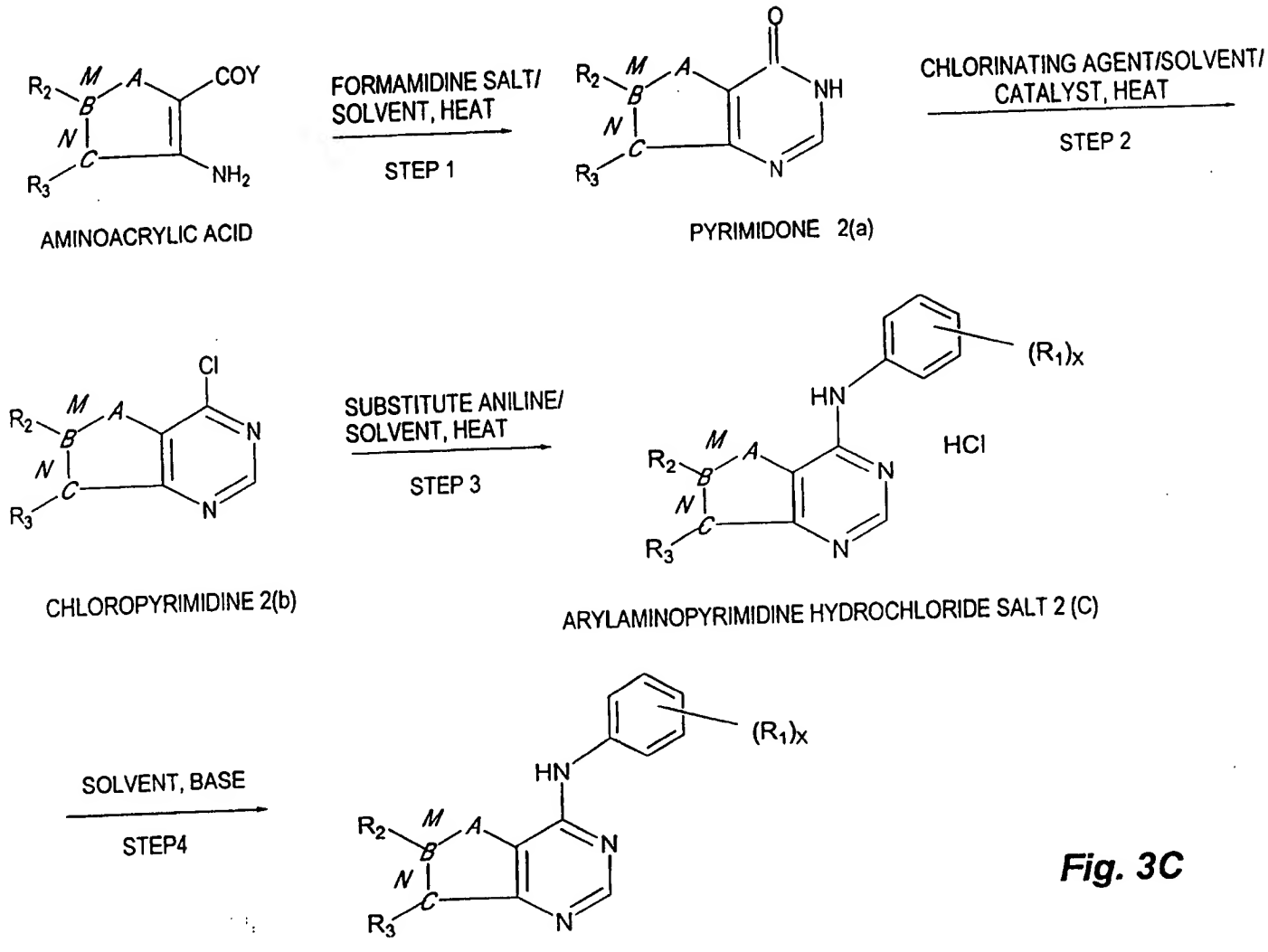
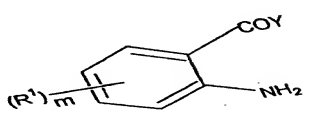
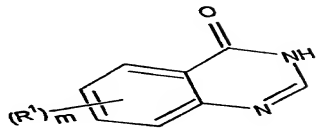

AMINOACRYLIC ACID
FORMAMIDINE SALT/ SOLVENT, HEAT
STEP 1
PYRIMIDONE 2(a)
CHLORINATING AGENT/SOLVENT/ CATALYST, HEAT
STEP 2
CHLOROPYRIMIDINE 2(b)
SUBSTITUTE ANILINE/ SOLVENT, HEAT
STEP 3
HCl
ARYLAMINOPYRIMIDINE HYDROCHLORIDE SALT 2 (C)
SOLVENT, BASE
STEP4
ARYLAMINOPYRIMIDINE 2 (D)

Fig. 3C



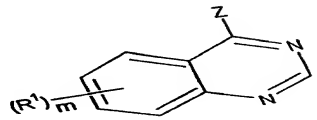
FORMULA I

Fig. 4a



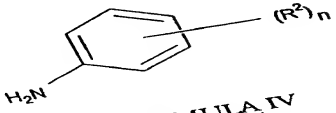
FORMULA II

Fig. 4b



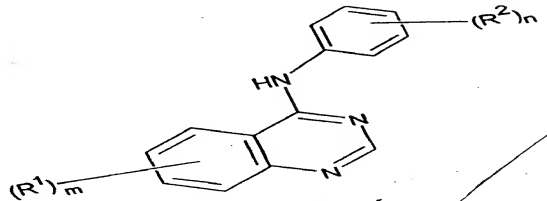
FORMULA III

Fig. 4c



FORMULA IV

Fig. 4d



FORMULA V

Fig. 4e